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THE PHILIPPINE
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B. MEDICAL SCIENCES

VOL. II DECEMBER, 1907 No. 6

THE ETIOLOGY OF MYCETOMA.

REPORT OF A CASE OF THE OCHROID VARIETY OCCURRING IN THE PHILIPPINE ISLANDS AND CAUSED BY A NEW SPECIES OF STREPTOTHRIX (*STREPTOTHRIX FREERI*).

By W. E. MUSGRAVE and M. T. CLEGG.
(*From the Biological Laboratory, Bureau of Science, Manila, P. I.*)

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CASE REPORT.
  Study of the amputated foot.
    Gross pathology.
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THE STREPTOTHRIX.
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INTRODUCTION.

Fully to appreciate the confusion which exists at the present time concerning the etiology of mycetoma, it is only necessary to consult the two systems of medicine which are now appearing simultaneously in the English language. J. H. Wright, in Osler's Modern Medicine, maintains that the black variety of mycetoma is a separate and distinct disease caused by a *Hyphomycete* which he cultivated from the black granules taken from a patient encountered in the Massachusetts General Hospital, although the author states that the etiologic relation of the *Hyphomycete* remains to be established by animal experiment. Wright, in discussing the ochroid variety of the disease, says that "at the present time the ochroid or pale form of mycetoma must be regarded as actinomycosis of the part."

On the other hand, MacLeod, in Allbutt's System of Medicine, recognizes Vincent's *Streptothrix maduræ* as the cause of the ochroid variety and states that it is a *Streptothrix* closely allied to, but differing from *Actinomyces.* This author, referring to the black type, is of the opinion that the fungus is a degenerate variety of the one causing the ochroid form of infection, rather than that it is a distinct species. MacLeod therefore considers the two varieties of the disease as being different stages or types of the same infection and of a single etiology. Other recent observers believe that the condition may be the result of the action of several closely allied species of *Streptothrix.*

We have isolated by culture and demonstrated conclusively the etiologic rôle of a new species of *Streptothrix* in a case of the ochroid variety of the disease, as will be seen in the following report, and have written to various colleagues for cultures of other organisms which have been isolated from types of the disease. As soon as practicable we hope, by a comparative study of these cultures and by observing their action on monkeys, to determine the etiologic importance of the other cultivated species of the *Streptothrix.*

CASE REPORT.

Mycetoma, ochroid variety; duration three years; amputation of foot; recovery. Streptothrix (Streptothrix freeri sp. nov.) cultivated from the lesions and its etiologic significance established.

P. B. (St. Paul's Hospital, No. 3256).—A Filipina woman from the Province of Bulacan; age 30, married, with two healthy children 2 and 5 years of age; was admitted on August 5, 1907, to Musgrave's service in St. Paul's Hospital. She complained of a large and painful right foot and stated that the disease was of three years' duration. The family and previous history of the patient furnished no data of importance. The patient stated, with reference to the present disease, that about three years before admission, while she was doing laundry work in the river, a sliver of bamboo entered the flesh between the great and second toes of the right foot. The splinter was withdrawn and the wound then healed. After about one month, pain in the region of the injury developed and the wound again opened and discharged pus. Following the first lesion, other nodular lesions developed in various places in the foot, and then subsequently opened and discharged pus. The foot slowly grew larger until it attained its present size.

The patient, on admission, is a somewhat frail, anæmic woman, apparently about 40 years of age, although she states she is but 30. She says she is in good health, were it not for pain and soreness of the right foot and her inability to walk, because of the size and weight of this member. The heart and vessels,

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instances mycelial threads may be seen. The alteration in tissue in sections from many other places in the foot is so great as to render the differentiation of the various elements impossible.

The whole foot is practically a mass of degenerated tissue, débris, and fungus elements, in which an occasional, recognizable piece of nerve, bone, or tendon may be seen. There are areas of fixed tissue-cell proliferation, in places usually surrounding a partially occluded blood vessel or a granule of the fungus mass. Evidences of repair of tissue or of acute inflammation are almost entirely absent. The *Streptothrix* is found in the sections in the form of granules and of occasional mycelial threads, both of these types being evident in masses of débris lying in somewhat definitely outlined cavities, and again among better preserved tissue cells. The granules vary in size from about 25 μ to 5 millimeters or more, and although they usually are oval or round in the smaller masses, they are of dumb-bell or more irregular shapes in the larger ones. The smaller granules, when appropriately stained, appear as conglomerate masses of mycelia, like puffballs, in which degenerated cells, crystals, and unstainable débris may be embedded; the margins of these granules often show a more or less definite radiation of fibers from the central mass, coccoid and other irregular forms being rare. The picture is somewhat different in the larger granules. These, when they are cut through in the process of sectioning, show three fairly well-marked zones. The outer of these is made up largely of radiating, mycelial bodies of varying shapes, some appearing as hyphæ, others as twisted and bent mycelia, and in some sections a more or less definite "club" formation may be noted. The middle zone is composed of irregularly interwoven mycelia with a few irregular forms, a mass of poorly staining material, degenerated cells, and a few crystals is embedded in it. The third or inner zone seems to resemble a cavity filled with granular and hyaline matter, broken-down cells and irregular forms of the *Streptothrix*; these latter being of several shapes, of which the bacillary and coccus-like predominate. (Pl. 3, fig. 1.)

THE STREPTOTHRIX.

Morphology.—A *Streptothrix* answering to the following description was found in large numbers in the discharges from the open sinuses and the various tissues of the diseased foot after amputation; it was cultivated and the disease was produced in monkeys' feet by inoculation with cultures and scrapings from the amputated member.

The discharges and scrapings from the cut surfaces of the foot, on microscopic examination, show the distribution of the *Streptothrix* to be very general and not confined to any particular tissue, or even to the sinuses and cavities. The organism is easily recognizable in fresh coverglass preparations. It occurs in several forms. The most important of these are granules of varying sizes, the larger ones being of the "fish roe" type discussed by several authors. These granules are of a dull, yellowish-white; the yellowish color being more pronounced in the larger and hardly noticeable in the smaller ones. They are of a tough, dough-like consistency, they may be washed and handled with impunity, and show evidences of considerable elasticity. They vary in diameter from 0.25 to 5 millimeters or more, are of diverse shapes, the very small ones usually being oval, the conformation differing as they grow larger. When pressed out by a cover glass and examined under a low power of the microscope, they somewhat resemble in appearance similar preparations of *Actinomyces hominis*; however, the ray formation is a much less distinctive feature and the "club" appearances are absent, or much less noticeable. Under the high powers of the microscope the smaller granules are seen to be made up of a mass of interwoven, fungoid filaments with a more or less definite picture of radiation of the filaments at the margins. The central portion in larger and older granules contains fewer of the filamentous forms, but there are numerous, bacillary and coccus-like varieties together with crystals, bright, shining, small, round bodies, and débris, the whole making up a more or less dense mass which, as it were, constitutes a cave for the granule. Outside of this mass there is a zone of interlacing filaments, in which cells and débris are embedded and, finally, the outer fringe is made up largely of terminal branches, loops, and hyphæ, which are forms of the fungus. Here, as is the case in the smaller nodules, there is some attempt at radiation and in certain of the specimens definite "clubs" may be seen. The *Streptothrix* likewise often appears in the fresh material as a somewhat skein-like mass of filaments or mycelia, with hyphæ and occasional round forms. These filaments are often found free in the discharge, and because of their loose structure are good specimens for examination. Still other types of the *Streptothrix* are sometimes present. These consist of a single filament, or of a few small, round, glistening bodies lying free in the secretion. The nature of these small, round, refractive bodies is not entirely clear. They are found free, bound up in meshes of mycelia, and what appear to be similar structures are sometimes observed inclosed within large fixed tissue cells, this process of inclosure apparently being one of phagocytosis. Yellowish crystals of various sizes and shapes, the products of parasitic activity, occur in the discharge in small groups, or singly. A pigment of the appearance of hæmoglobin may also be seen.

The individual elements of the *Streptothrix* give interesting pictures when they are examined with the highest powers of the microscope. The hyphæ-like forms seem to be made up of a delicate membrane, covering a homogeneous and unbroken core; in the older, mycelial types the outer membrane appears to be irregular in thickness and density and absent in places; the inner portion is also frequently and irregularly broken, and thus the appearances of bacillary and ovoid or coccal sections is produced. Branching forms are plentiful, the branches first apparently appearing as buds, which grow to hyphæ from the side of the mycelial thread. True dichotomous division has not been observed. The small, round bodies mentioned above often appear to have a double containing wall and they vary in diameter from 3 to 10 μ.

Staining.—The *Streptothrix* takes the majority of the ordinary stains in preparations of fresh material; it is stained by the Gram-Weigert method, and its acid-fast and alcohol-fast properties are quite marked, particularly in the older filaments.

The Ziehl-Neelson-Gabbet method for staining the tubercle bacillus is also very satisfactory for *Streptothrix freeri*, the fungus elements assuming the same bright red color which is imparted to tubercle bacilli. The structure of the granules becomes fairly apparent by the various methods of staining and the specimens confirm the observations already recorded when discussing the fresh materials.

Stained specimens which show the individual elements give some interesting results. Some specimens, when they are colored by the method used for tubercle bacilli, contain so many of the bacillary forms which take the red stain and which closely resemble *Bacillus tuberculosis*, that we at first thought the latter organism was also present in our cultures. However, further study has shown these, as well as the coccal and other irregular forms, to result from the breaking up of the older mycelial filaments. These bodies which resemble tubercle bacilli may sometimes be seen in the filamentous mycelia, still surrounded by the unbroken sheath.

Cultures.—Cultures on agar and glycerin agar, from the open wounds before amputation and after the foot had been dressed for twenty-four hours in a wet bichloride dressing, remained sterile. The *Streptothrix* was cultivated without difficulty from the sinuses and tissues of the amputated foot and no bacteria were present except when the cultures were taken from those surfaces contaminated by the knife in opening the foot; in the latter instances saprophytic bacteria were also present.

Lesions from which the *Streptothrix* was obtained in pure culture were produced in monkeys after the inoculation of fresh material. The microörganism is aërobic and it grows with greater or less readiness upon most of the ordinary laboratory media. Cultures upon media containing sugar or glycerin and upon potato have some color, which varies in intensity.

A profuse growth appears in a few days in certain media, but in others the development is much slower. Potato, sugar containing media, and glycerin agar at 37° C., are best adapted to the growth of the fungus, but a slower development takes place even at ordinary room temperature (30° C.). The organism is an obligate aërobe.

Potato.—Growth becomes visible after twenty-four hours and after seventy-two becomes luxuriant. The colonies, when they first become apparent, are small, discrete and slightly raised, and have a delicate, pink color. As growth proceeds they become larger at the base and raised to a height of at least 0.1 millimeter. The upper portion of the colony appears as a short shred which often folds upon itself, giving it the appearance of being umbilicated. The whole inoculated surface finally changes to a heaped-up mass which has a yellow-ocher color in the center, with a pinkish or white periphery. The medium becomes darkened and moist. The growth may be lifted in heaps on a platinum loop. Desegmentation occurs when it is placed in water and small, flat particles, from delicate pink to a yellowish-brown color and which resemble dry bran, float upon the surface; on shaking the vessel the particles adhere to the sides of the vessel. (Pl. IV, fig. 2.)

Glycerin agar.—Growth appears after three days; later it becomes heaped up, the medium remaining moist. The colonies at first are discrete, but they soon become confluent, but not diffuse; they are raised and in some instances have the umbilicated appearance observed on potato. As the cultures grow older, they to some extent resemble those of the tubercle bacillus on the same medium. A yellowish pigment is produced, more pronounced in the central portion where the growth is more luxuriant; with a pink to pinkish-white periphery. The growth may readily be peeled from the surface of the medium, and when observed in bulk it has a moist appearance. No pigmentation of the medium occurs. (Pl. IV, fig. 1.)

Agar-agar 1 per cent acid and 1 per cent alkaline to phenolphthalein.—The growth after four days' incubation appears as a smooth, white and glistening, very slightly raised mass. The colonies do not develop the central pit, thus differing from their appearance on potato, glycerin agar and the media containing sugar, neither do they produce the pigment which is characteristic of growth on the latter, but on the contrary they first assume a porcelain-white color and later develop a delicate, diffuse pink. There is no pigmentation of the medium.

Loeffler's blood serum.—Growth on this is much slower than on other media. The pigmentation and growth are very similar to those on plain agar-agar. After two weeks' incubation no change in the medium occurs.

Glucose agar stab.—Colonies do not develop along the track of the needle, but they grow luxuriantly on the surface of the medium forming a heaped-up center, with a wrinkled periphery. The center is yellow in color, the periphery pink to pinkish-white. No pigmentation of the medium occurs. The growth on this medium shows the aërobic tendencies of the microörganism.

Gelatin.—This medium is not liquefied; growth is similar to that on glucose agar.

Bouillon, alkaline and acid.—Floating, flat particles appear on the surface of the medium after three days' incubation. These particles, when the tube is shaken, adhere to its sides. On further incubation, a sediment in the form of a tenacious mass settles to the bottom. The medium does not become cloudy, but on long incubation it changes to a darker color.

Alkaline litmus-milk.—Growth appears after three days on the surface of the medium in the form of dry, flat particles, which later become confluent, forming a heaped-up, yellowish mass. A tenacious sediment forms at the bottom of the tube; in this portion of the medium the milk is gradually decolorized; however, it does not become red. The milk is not coagulated.

Lactose-litmus agar stab.—The growth on this medium is similar to that on the glucose-agar stab and it produces no change in the reaction of the medium.

Potato infusion.—The growth is similar to that on bouillon; however, no pigment is produced and the medium is not altered.

Relation of growth to temperature and resistance to heat.—Growth was observed both at room temperature and at 37° C., but under the former condition it was much slower and less pigment is produced than at the temperature of the incubator. No growth occurs in cultures after exposure to 70° for 15 minutes.

ANIMAL EXPERIMENTS.

The series includes forty monkeys (*Cynomolgus philippinensis* Geoff.), guinea pigs, rabbits, dogs and pigeons. In nearly every instance lesions characteristic of mycetoma were artificially produced by intraperitoneal inoculation, with the exception of the rabbits and pigeons. Three typical examples of Madura foot developed in monkeys after injection of the organism into the foot. (See Pl. I, figs. 2 and 3.) On the other hand, in no instance was a progressive disease produced by *subcutaneous* inoculation in other parts of the body.

INTRAPERITONEAL INOCULATIONS OF MONKEYS.

Monkey number 3268.—Inoculated into the abdominal cavity with material from the original case. After five days, a small tumor was located above the bladder in the left inguinal region and three days later the animal was found to be in a dying condition. It was immediately killed and autopsy performed.

Autopsy.—Heart and lungs appear normal; no fluid in either cavity. Spleen, liver and kidneys normal, some fluid found in the abdominal cavity. A tumor 2 centimeters in diameter is present just above and to the left of the bladder. This tumor is surrounded by adhesions and is adherent to the omentum, abdominal wall and intestine, it is extremely firm in the center, with a surrounding œdematous coat. The cut section shows several small sinuses, from which small quantities of pus may be expressed. Small, distinct, refractive granules can be seen macroscopically, none larger than a pin head. Smears show the presence of a *Streptothrix* and *S. freeri* was reclaimed in pure cultures.

Monkey number 3269.—Inoculated with the material from the previous animal. After six days the animal showed a disposition to sit in a cramped position. On palpation a nodule, about 2 centimeters in diameter, which seemed to be attached to the abdominal wall was located in the abdominal cavity. Eight days later the animal was found to be in a dying condition. It was killed and autopsy performed immediately.

Autopsy.—Lungs, heart, liver and kidneys normal. Some fluid is found in the abdominal cavity. Above the bladder, and occupying the entire abdominal cavity there is a firm mass composed of necrotic tissue and compact adhesions of the intestinal coils to the abdominal wall and to the omentum. A cut section shows numerous sinuses surrounded by thickened walls, in some instances these sinuses have burrowed through the walls of the intestines and in others into the wall of the abdomen. A thick, cream-like discharge in which small, glistening grains or granules are visible, can be pressed from the cavities. Pure cultures of the *Streptothrix* were obtained.

Monkey number 3307.—Inoculated with 0.2 of a potato slant culture obtained from monkey number 3268. After ten days a small nodule could be felt above the bladder in the left inguinal region. Several days later, multiple nodules developed, and ten days afterward the animal died.

Autopsy.—Several distinct nodules, which contain thick, cream-like pus are present. Anterior to, and a little above the bladder there is a tumor 2 centimeters in diameter, bound by adhesions to the mesentery of the descending colon, to coils of the small intestine and to the abdominal wall. On section of the tumor, a heavy, cream-like pus is discharged from the center; the surrounding mass is composed of a necrotic material containing small sinuses filled with pus. Smears show numerous growths of the *Streptothrix.*

Monkey number 3308.—Inoculated with 0.25 of a potato slant culture. The animal died ten days later, the lesions being similar to those found in animal number 3307.

Monkey number 3309.—Inoculated with 0.25 of a potato slant culture in the abdominal cavity. The animal is still living, thirty days after the inoculation. On palpation, a small nodule can be distinguished in the lower portion of the abdominal cavity.

INTRAPERITONEAL INOCULATION OF GUINEA PIGS.

Guinea pig number 3313.—Inoculated with a small amount of the culture grown from monkey number 3269. The animal died seven days after the inoculation.

Autopsy.—Ten cubic centimeters of a clear fluid are found in the abdominal cavity, the heart is slightly dilated, the lungs congested, the diaphragm shows numerous, small, superficial abscesses varying in size, the largest not more than 2 millimeters in diameter. The liver is intensely congested and contains similar abscesses, and they are also found in the omentum, mesentery and spleen. Pure cultures of the microörganism were obtained from the abscesses.

Guinea pig number 3314.—Inoculated with a small amount of the culture grown from monkey number 3269. The animal died ten days later.

Autopsy demonstrates lesions identical in character with those found in guinea pig number 3313.

Guinea pig number 3323.—Inoculated with 0.5 cubic centimeter of an emulsion of material from abscesses from monkey number 3269. At the present time, one month later, the animal is still living and seems to be in good health.

INTRAPERITONEAL INOCULATIONS OF DOGS, RABBITS AND PIGEONS.

Three healthy dogs weighing about 8 kilograms each were used in these experiments. In every case lesions identical in appearance with those described in the monkeys developed.

Ten rabbits and three pigeons were inoculated with fresh material from lesions and with cultures of varying ages, but in all cases the results were negative.

SUBCUTANEOUS AND INTRAVENOUS INOCULATIONS.

Subcutaneous inoculations into a large number of rabbits, guinea pigs, dogs and monkeys all gave the same results. Small nodules developed only at the points of inoculation; these soon underwent resolution and finally healed. In many instances pure cultures of the *Streptothrix* were obtained from the discharges.

Cultures and emulsions of the fresh material were used for intravenous inoculation of rabbits, but all the results were negative.

INOCULATIONS INTO MONKEYS' FEET.

Monkey number 3266 was inoculated with fresh material, in the left foot, after incision and scraping the metatarsal bones. Sixteen days later a marked swelling of the foot developed, and after twenty days, numerous, suppurative lesions appeared between the toes, on the ball of the foot, and on the heel. The animal died of malnutrition.

Section of the foot.—A cut section of foot showed a slight necrosis of the tarsal bones and numerous sinuses, containing pus, surrounded by necrotic material extended throughout the organ. A pure culture of the microörganism was isolated from the discharge.

Monkey number 3310.—Inoculated with 0.25 of a potato slant culture in the ball of the foot. After ten days a marked swelling developed, this afterward suppurated and broke down, discharging a viscid, cream-like pus, containing a quantity of the *Streptothrix.* At present the animal is still living and shows a typical Madura foot. (See Pl. I, fig. 2.)

Monkey number 3311.—Inoculated with 0.25 of a slant potato culture in the ball of the foot, the animal is still living and presents the same characteristic lesions as does monkey number 3310. (See Pl. I, fig. 3.)

HISTORY AND LITERATURE.

Notwithstanding the inaccessibility of many of the older articles in the original, we have been able to study practically all of the important discussions of this subject, and from these, as well as from other references, Miss Polk, librarian of the Bureau, has been able to compile a fairly complete bibliography. No article of great importance, except from an historical standpoint, existed before the observations of Vandyke Carter, which extended from 1859 to 1874. The greater part of the oldest literature is in the English Army Reports from India and therefore is not available to the general public.

Kämpfer (1712), according to Scheube, was the first European physician to mention the disease, which he discussed under the name *"Perical."* The same author states that Heynes (1806) reviewed the subject in his historic and statistical news of India. Both of these writers, and perhaps all others before 1840, confused Madura foot with elephantiasis and other independent diseases. However, to judge from the translation of some of the older native names it seems that it was considered to be something specific and definite by the natives of India. Scheube informs us that Brett (1840) was the first author to indicate that Madura foot is a specific disease.

During the ten years from 1840 to 1850, mention of the disease occurs several times; the following may be mentioned: Gill (1842) reported it from Madura; Godfrey (1844) from Bellary; Colebrook (1844) from Madras; Gunther (1844) from Coddapah. Eyre (1848) reported cases from Bellary and according to Hirsch made the first collection of the literature and he as well as Godfrey and Colebrook[1] (1850) gave a general description of the disease, the latter indicating its specific character. Scheube states that Ballingal (1855) reported one case and first described the infection in detail as being of a parasitic nature. A careful description was also given by Eyre (1860), by Collas (1861), (Hirsch) and Biddle (1862), who reported cases, and by

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[1] Military Med. Reports, Madras (1850).

Hirsch[2] (1863 and 1886), who gave a general and historical review. Coquerel (1866) published a report of one case of mycetoma. He recognized and illustrated the fungus elements present in the lesions. Hirsch also states that Minas added several cases from Hindostan to the literature, and Atchison gave several instances of the disease from Jhelm in the Punjab. According to Le Dantec, Duval, Berénger-Férand, and Carpot encountered cases which they mentioned in the literature in Senegambia, and Legrain in Algiers. Moxon and Hogg[3] note fungus-like filaments in the granules in an instance of *ochroid* mycetoma. Bristowe[4] made a study of the melanoid granules in one case. He recognized the presence of a fungus which he considered to be identical with that discovered by Carter. During[5] and Rochefort,[6] according to Scheube, also mention the disease. Hogg[7] studied a case of the melanoid type and recognized the presence of a fungus in the black granules. The patient was cut on the foot by a stone six months before the development of the symptoms. Berkeley[8] cultivated a fungus from specimens sent to him by Carter and named it *Chionyphe carteri*, but subsequently denied the etiologic significance of this parasite. Fox[9] reported cases of the disease and in discussing the etiology questioned the importance of the fungoid elements which had been described in this connection.

Lewis and Cunningham[10] after a study of the tissues obtained from a case of the infection came to the conclusion that mycetoma was "essentially a degeneration of the fatty tissues, independent of the local presence or influence of any parasites whatever."

Regardless of the previous history of mycetoma, we owe the establishment of the condition as an independent and specific disease to which this author gave the accepted name, mycetoma, to the researches of Vandyke Carter, who began the work in 1859 and who finished his publications by a monograph[11] in 1874. In the first of his articles Dr. Carter strongly upholds the fungus nature of the infection and his publication is so complete as to leave but little to add, even at this day, excepting discussions concerning the etiology and histology of Madura foot. Carter states that in October, 1859, he examined an amputated foot immediately after operation and "found the clearest possible evidence of the true fungus structure of the black (melanoid) particles now well enough known." This observation was afterwards invariably confirmed by himself and, according to his own statement, also by Bristowe.[12] This discussion concerns the *black* variety of the infection.

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[2] *Arch. f. path. Anat.*, Berl. (Virchow's Arch.) (1863), **27**, 98, and Handb. der historisch-geographischen Path. (1886).

[3] *Trans. Path. Soc.* Lond. (1870).

[4] *Trans. Path. Soc.* Lond. (1871) (Vincent gives it 1881).

[5] Eulenburg's Real-Ency. d. ges. Heilk. (3d ed.) **14**, 203.

[6] *Arch. d. Med. Nav.* (1876), **25**.

[7] *Med. Times and Gaz.* (1871), July 22, 93, and *Trans. Path. Soc.* Lond. (1872), **18**, 294.

[8] *Med. Press* and *Cir.* (1876), 465.

[9] *Trans. Path. Soc.* Lond. (1870), **21**, 411, and (1871), **22**, 320; *Lancet* (1876), 190.

[10] 11th An. Rep. San. Com. Govt. India, Calcutta (1875–88).

[11] On Mycetoma or the Fungus Disease of India, Lond. (1874).

[12] *Trans. Path. Soc.* Lond. (1871), **22**.

In November, 1859, Carter examined a foot before and after amputation, and found in it and in the discharges from the openings on the surface *"numerous minute, pale particles,* which also presented a decided fungus structure." The fungus-like appearance of these bodies was subsequently confirmed, Carter says, by the members of the Medical and Physical Society to whom he showed his specimens at one of their meetings. Therefore, Carter concluded that although there were apparent differences between the fungi of the pale and black types, and that those of the former were very peculiar structures, *Chionyphe* could be seen to spring from the particles (sclerotia) obtained from them as well as from those of the *black variety,* and it was his opinion that the two forms are but varieties or different stages of the same disease.

After further discussion Carter concludes as follows:

> "The foot disease of India is not a carious, strumous, or myeloid, or any like affection; but a veritable parasitic disease, due to the growth and extension, within the tissues of the human foot, of an indigenous mould or fungus of true plant nature."

It is apparent from a review[13] of his monograph[14] that Dr. Carter was a pioneer in his observations in the establishment of the cause of the disease, and in maintaining the identity of the two varieties. However, the reviewers of his monograph criticized Carter's conclusions and pointed to the lack of evidence submitted by him in support of the parasitic nature of the so-called fungus.

Corre (1883), in reporting the notes of Dr. Collas, states that the latter observer considered *Epulis gravis,* the pseudo-cancer of the inferior maxilla found in Pondicherry, to be identical with mycetoma. Vincent, Roux (1888) and Brun (1893) describe the early symptoms of the disease; Lebroux (Bordeaux 1887) in a thesis (not available to us) discussed the whole subject of mycetoma and Bassini (1888) reported a case (the first in Europe) of melanoid mycetoma which occurred in Italy. He recognized the fungus nature of the granules, the radiating type of the filaments and the irregular swellings in segments. He considered them to be somewhat of the nature of the *Aspergilli* or *Mucorini.* Bassini's patient pricked his foot in an ox stall; the wound healed, but a tumor developed and by the seventh or eighth month the patient could not walk. The fungus was found in the typical tumor removed at operation, but attempts at cultivation were not successful.

The decade from 1890 to 1900 furnished a number of interesting contributions to the subject.

Hewlett (1892 and 1893) in his first article described findings obtained from the study of a preserved specimen sent from India and concluded that mycetoma is identical with actinomycosis. In his second paper he reports two cases of the ochroid variety, and reiterates his belief that the process is the same as actinomycosis.

Kanthack (1892 and 1893) studied sections from the prepared material obtained from three cases of the black variety; he noted the fungoid elements and although he "found it difficult to convince himself of their vegetable nature," he thought that they might be degenerating types of an organism similar to *Actinomyces.* Kanthack also examined sections from preserved material taken from twelve cases of the ochroid variety, and states that "the parasite of the ochroid variety agrees morphologically and structurally with the typical and perfect ray fungus, and there can be no reasonable doubt that both belong to the same botanical group." Kanthack concludes that "(1) the fish-roe masses are undoubtedly a form of *actinomyces;* (2) the black masses in their most perfect shape are also of this nature, but in a state of degeneration; (3) a degenerated form of the yellow variety is occasionally found, in appearance not wholly unlike these black masses, so that the relation between all these forms seems to be fully established."

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[13] *Lancet* (1874), 591.

[14] "On Mycetoma or the Fungus Disease of India, Lond." (1874), 113.

Boyce and Surveyor (1893 and 1894), in addition to a thorough discussion of the etiology of the disease with special reference to the difference between the two varieties, have given one of the most succinct and comprehensive general descriptions of mycetoma which we have seen. Their description is so lucid and brief that we quote from it as follows:

"The Madura foot of India is a very chronic affection, lasting in some cases twenty-five years. It is purely a local disease of the extremities, chiefly the foot, and generalization has not been observed. It usually occurs in people who go barefoot and are working in fields. In most cases it has been traced to some injury. The big toe is often affected at first. The disease stops at the ankle for a short time, then it spreads up to the knee, and eventually may even reach the thigh. One of us has had the opportunity of seeing a recurrence in the scar after amputation; this, however, must be very rare, and amputation affords complete relief * * * and is, indeed, one of the most successful operations in India. The foot * * * is greatly altered; it is enlarged, often many times the natural size. The overgrowth of the foot is irregular; the toes may become buried, * * * and the surface becomes studded over with mammillated, or even villous, projections. A large number of the mammillated projections mark the presence of sinuses, which pass deeply into the foot; and on section these may be seen to honeycomb it. From the opening of the sinuses a purulent or sanguinous discharge can be pressed out, and in this are found, in one series of cases, small particles of a light yellow color, which have been compared to fish roe; while, in the remaining cases, deep brown or black particles, resembling grains of gunpowder, may be seen. The disorganization of the interior of the foot becomes very complete in time; the bones undergo a rarefactive ostitis, and are ultimately absorbed, granulation tissue also invades the muscles and fat, and lends to their disappearance. Associated with the hypertrophy of the granulation tissue there may be considerable hyperplasia of the epithelium of the skin. This overgrowth gives rise to the mammillated and papillomatous projections previously referred to.

"The only difference which one at present finds, clinically and microscopically, in the numerous cases of Madura foot is the difference in the size and color of the particles. Carter termed that form of the disease in which the black particles were present the "melanoid" variety, while under the "white" or "ochroid" were grouped those cases in which the fish-roe like bodies were found. It will be understood that only those cases of Madura disease in which the particles can be demonstrated macroscopically or microscopically will be admitted as genuine. The disease appears to be confounded with scrofula and various forms of elephantiasis, in the production of which probably other parasites play an important part."

Boyce and Surveyor examined, in a most careful manner, tissues from six cases of the melanoid and from fifteen of the ochroid variety of the infection. In an addendum to their article, they mention having studied "a large number of cases of the disease" including fifteen more of the melanoid type. The authors "bring forward reasons for believing that there are at least two distinct fungi—one, a very highly organized species, the other a very delicate and lowly organized type, presenting very many of the characters of *Actinomyces*."

By the use of sodium hypochlorite as a clearing agent in the case of the black granules, and logwood as a stain, they very clearly showed the fungus nature of the granules and their illustrations are very clear. They write, while discussing the granules of the ochroid variety, that whereas in some respects these granules resemble those of actinomycosis "it would be rash to state that the white particles are always actinomycotic." Boyce and Surveyor failed to grow the parasite upon artificial media. Their article is interesting and valuable and may be commended to anyone who wishes to review the subject.

In 1894 Boyce states that the organism of the black variety is a richly growing fungus, which is rendered more visible by solutions of sodium hypochlorite; that of the white one is a more delicate organism, closely resembling that of actinomycosis. This author had secured additional data by means of cultures of the parasite. Agar tubes, inoculated by friends in India and forwarded to him, were usually contaminated by other organisms, but one tube which contained the white variety was, upon its receipt by the author, free from them.

Microscopic examination of this culture showed colonies of a thick mycelium, firm and difficult to tear apart; the mycelium contained vacuoles, and its membrane was very thin. Club-shaped forms were not found and dichotomous branching was present. Subcultures were secured on grape sugar, agar and glycerin agar at 35° to 37°. Growth was very slow, much more so than in the case of *Actinomyces*. Colonies having a peculiar appearance slowly developed. These colonies had odd markings. At first quadrates appeared, following them there were more divisions, until finally they were separated into many segments, no fusion of the colonies being apparent. Growth is almost entirely inhibited on potato or on acid or alkaline bouillon. There was no formation of pigment on potato or agar, the growth therefore differed from *Actinomyces*. A comparison between the cultures and particles taken directly from the foot showed great similarity.

The author conducted a number of experiments with animals and demonstrated that subcutaneous inoculations of rabbits, guinea pigs, monkeys and rats always gave a local reaction which took the form of a tumor, increasing in size during two or three weeks, after which time it became smaller and harder. Boyce concluded that the fungus which he described was a new variety causing the white type of Madura foot. Boyce and Surveyor (1894) in a third article, continue their discussion of the cultivation of the parasite of Madura foot. Their material was inoculated into culture media in India and forwarded to them in England. The following quotation from their later work has an important bearing on the history of the subject:

"This organism grows slowly on most of the nutrient media we have tried. In gelatin and broth it does not seem to grow at all, but cultures taken from these media begin to grow when transferred to potato or glycerin-sugar-agar. On these media the growth does not appear to make any progress for about a week, but after that the margin around the inoculated area shows slight projections, which thicken gradually and give the growth a radiate appearance. Minute colonies are found around the central growth, and these show a cross-like depression, which becomes more marked as they increase in size. The growth is firmly adherent to the nutrient medium at the margins, while the central part is loosely attached. The color is dull white or slightly pinkish."

"The culture is brittle and crumbles easily. It grows best at 39°. It is aërobic. "In an atmosphere of hydrogen the growth is excessively slow. In this respect it differs from actinomyces which grows easily in an atmosphere of hydrogen."

The organism was readily stained by aniline dyes and by Gram's method. The filaments were long and slender, no septa could be distinguished and dichotamous division was not observed. Inoculation experiments with the cultures were not made.

Le Dantec (1894) reported cases from Africa. According to Scheube, Le Dantec cultivated *Staphylococci*, *Streptococci*, and short bacilli from the truffle-shaped bodies. In bouillon the bacilli grew into long filaments without ramification. Cultures on solid media were only obtained by transplants from bouillon and not directly from the material. All cultures gradually assumed a reddish-rust color and rabbits and guinea pigs were not susceptible to infection by them.

Hatch and Childe (1894) report a case of the black, or a combined black and ochroid variety, in an African negro. The disease primarily involved the knee, and subsequently extended to the abdominal wall and internal abdominal structures, particularly the lymphatics. The fungus failed to grow on bouillon or glycerin agar. The descriptions and illustrations of this case show characteristics making it probable that the disease was actinomycosis rather than mycetoma.

Vincent (1894) reported a case of ochroid variety and cultivated a *Streptothrix* from the lesions. The granules resembled *Actinomyces*, being yellowish-white in color and insoluble in potash and in acetic acid; when stained by Loeffler's method or by fuchsin and enlarged 400 to 500 times, they were seen to be made up of filaments and mycelial débris. The peripheries of the granules showed filaments of radiating types. Vincent considered the parasite to be a *Streptothrix* and named it *S. maduræ*. The filaments when examined under the high powers of the microscope were shown to contain elements of varying size, some being so small as to resemble micrococci. Scheube's abstract is so satisfactory that we have quoted it, after comparing the original.

The material used by Vincent for the preparation of cultures was taken by him direct from the diseased tissue. "It exhibited but little growth in bouillon, but infusions of hay and straw (15 grams to 1 liter) not neutralized, and therefore with an acid reaction were found to be suitable nutritive media. The same holds good for meat soups, to which yellow turnips, carrots, and more especially potatoes (20 grams to 1 liter) have been added, after previous filtration and sterilization. The temperature should be about 37° C; if it is over 40°, growth stops. The cultures, which are placed in wide tubes or Ehrlenmeyer's flasks, to which air is accessible, exhibit small, gray flakes, round or flat, on the fourth or fifth day; these cling to the wall and bottom of the tube, and after from twenty to thirty days attain the dimensions of a small pea. Some of the flakes exhibit a brown tinge in the center, while others that lie near the surface of the liquid become pink or red after a month or two. The nutritive fluid never becomes cloudy, for most of the flakes lie at the bottom, where they form a covering which exceeds 0.5 to 1 centimeter in thickness. The nutritive fluid, previously acid, in time assumes an alkaline reaction, and becomes pale-blue.

"In ordinary gelatin the *Streptothrix maduræ* only exhibits a weak growth of white colonies along the inoculative puncture on the surface. Vincent considers that the best solid nutritive soil is 100 cubic centimeters of an infusion of hay or potatoes, with the addition of 9 grams of gelatin, 4 grams of glycerin, and 4 grams of glucose, neutralized and sterilized in the usual manner. The *Streptothrix* does not liquefy the gelatin. On this soil fine, luxuriant, round, glazed colonies of a white or pale yellow color are obtained, which later on often assume a pink or even red color. If very many colonies form on the gelatin they remain small, but if fewer are present they attain almost the size of a pea. They then resemble an inoculation pustule sunk in the middle, and are of a white color, the margins assuming a red tint. Old cultures fade and become dull white. The colonies are of a horny consistency, and cling very firmly to the nutritive media.

"The parasite grows fairly well on milk without coagulating it, though it becomes slowly peptonized. On eggs and serum, however, it does not flourish at all.

"On potatoes, from the fifth day, and at 37°, small, uncolored, or whitish prominences are seen, which assume a pale, reddish color after a month. This gradually deepens and becomes sometimes a bright pink, sometimes orange-color or red, sometimes a fine, dark red. This occurs particularly when the potatoes have a strong acid reaction, whereas on some potatoes there is no coloring at all. On carrot media the red coloring is more pronounced than on potato.

"Some colonies are covered by a fine, white dust which consists of spores.

"The parasite is aërobic. The parasite stains well with basic aniline stains, more faintly with safranin and eosin. It can also be stained according to the methods of Gram and Weigert. A solution of iodine stains it yellowish-brown, while hæmatoxylin causes it to assume a violet color.

"The fungoid filaments are finer in the cultures than in the little bodies described above; their breadth does not exceed 1 μ. The irregular swellings and contractions observed in the little bodies are not observed in the cultures, which, however, when two weeks old often exhibit a series of spores at the end of the filaments. They are ovoid, light refracting and about 1.5 μ in breadth and 2 μ in length. They stain well with aniline dyes and according to Gram's method. They exhibit very little resistence to heat; they are killed in three minutes at 85°, and in five minutes at 75°. The cultures that do not carry spores die in three to five minutes at 60°.

"The formation of spores takes place best where the mycelium comes into contact with the air, and this is the case in fluid mutritive media as well as on potato. Cultures with hay broth are the most suitable for this purpose.

"One may actually see the spores develop in fresh broth and in suspended drops.

"The cultures are very resistant to desiccation; even after a period of twenty-one months they exhibited a capacity for development.

"The *Streptothrix maduræ* does not prove pathogenic to animals (rabbits, guinea pigs, mice, cats). Bocarro's experimental transmissions with fresh material of both varieties were negative in rabbits as well as in dogs. Nocard also inoculated with cultures guinea pigs, rabbits, pigeons, hens, dogs and sheep, by intravenous, subcutaneous and intraperitoneal inoculations, with negative results in every case.

"By means of parallel cultures arranged and kept under exactly the same conditions, Vincent confirmed the difference between the *Streptothrix maduræ* and the actinomyces, as shown on the table.

"Boyce and Surveyor likewise succeeded in cultivating a streptothrix in a case of the white variety; it exhibited a condition similar to that cultivated by Vincent, but no ramifications and spores could be discovered.

"Legrain could not cultivate colonies in infusions of hay, but succeeded in doing so on gelatin with malt and peptone. The colonies were round, prominent, glazed, very adherent to the lower surface, at first pale, then golden-yellow, and soon after became of a vivid red color and finally became decolorized.

"In the nodules which exhibited suppurative degeneration, Vincent found *Staphylococcus pyogenes albus* and *aureus* in addition to the parasites described."

Vincent gives the following comparative table of the cultural characteristics of *S. maduræ* and *Actinomyces:*

TABLE NO. I.—*Comparison between S. maduræ and Actinomyces.*

No.	Cultures or inoculations.	Streptothrix maduræ.	Actinomyces.
1	Peptonized beef tea	Moderate growth	Luxuriant growth.
2	Sterilized infusion of hay or straw.	Principal nutritive soil; growth rapid (4 days) and luxuriant.	No growth.
3	Ordinary peptone-gelatin	Does not liquefy	Liquefies.
4	Gelatin with infusion of hay.	Very quick growth; the culture becomes pink or red on the surface.	Whitish, very weak culture.
5	Glycerin gelatin	Colonies which at first are white, then pink or red, and umbilicated.	At first white, later on grayish spots; folded.
6	Potato	Bright pink, vivid or dark red culture; does not brown the substratum.	Close, wart-like yellow and white, black-edged colonies; potato becomes brown.
7	Cabbage, yellow turnip, carrot.	Growth	No growth.
8	Serum	No growth	Growth.
9	Egg	do	Do.
10	Cultures in areas deprived of air.	do	Facultatively anaërobic.
11	Inoculations	Not transferable to any animal	Transferable to rabbits, guinea pigs, calves.

S. maduræ according to Vincent differs culturally from the fungi isolated by Nocard,[15] Almquist,[16] and Eppinger.[17] The Nocard organism develops rapidly in peptone-bouillon and it does not grow in hay infusion. The cultural characteristics upon agar and potato present no analogy. Nocard's organism is inoculable in guinea pigs.

Vincent also discusses the question of the identity of actinomycosis and mycetoma and notes the following differences: Potassium iodide does not give the same positive results with Madura foot as it does with actinomycosis. Different results are obtained with the two parasites, not only morphologically but also in cultures in various media and in inoculation experiments.

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[15] *Ann. d. l' inst. Pasteur* (1888), 2, 293.

[16] *Ztschr. f. Hyg. u. Infectionskrankh.*, Leipz. (1890), 7, 189.

[17] *Wien. Klin. Wchnsch.* (1890).

Vincent and Gémy (1896) mention a second case of ochroid variety and the cultivation of the same *Streptothrix* already reported by Vincent. Preliminary mention was also made by these authors at the Congrés de Dermatologie et de Syphilis in Paris on April 25, 1892.[18]

Shattuck (1898) reported to the Pathological Society of London a case which he designated as *Mycetoma papillomatosum.* The skin exhibited a pronounced papillomatous condition, and secondary papillæ with sinuses and fungi were found within the larger growths. Maitland (1898) reported a case involving the thigh and abdominal wall of a man, and Smyth (1898) published one involving the tissues of the neck.

Plehn, according to Scheube, observed three cases in Suahelinese on the Tanga coast; however, probably these had been carried to that point from India. Vincent mentions the fact that Layet encountered the disease in Chile and Crookshank (1897) discussed the infection after collecting data obtained by the examination of a specimen sent from India. Boccaro (1893) analyzed one hundred cases, and in seventeen instances he saw evidence of a prick by an acacia thorn. In many of these cases the thorn itself was found. The same author also reports a case of mycetoma involving the tissues between the vertebral column and the scapula. Descriptions of the infection are also given by Delbanco (1897 and 1898); by Huntly (1890 and 1899), Kobner (1891), Paltauf (1894), Ruelle (1893), Shah (1893) and the pathology is fully outlined by Unna (1897).

The first case in America was reported by Kemper and Jameston,[19] but the article is not available to us.

Adami and Kirkpatrick[20] studied a case of the ochroid variety in Canada and considered the granules to be identical in appearance with those from actinomycosis, and Hyde, Senn and Bishop (1896) reported one from Chicago. In the latter instance the fungus suggested *Actinomyces.* No club-shaped forms were seen, and in sections the central portion of the granules stained faintly with hæmatoxylin. Under high power, delicate, radiating filamentary threads were observed in the masses. Delbanco, (1897) (according to Scheube) who studied materials sent him by the authors reporting this case, concluded that it was actinomycosis and not Madura foot. The record of Pope and Lamb's case (1896) is not available to us. Arwine and Lamb[21] (1899) mention another case in a native of Texas. Dr. Carroll who examined sections from the specimen considered the disease to be actinomycosis.

J. H. Wright[22] (1898) reviewed some of the literature of the subject and reported one case of the melanoid variety from the service of Dr. Beach in the Massachusetts' General Hospital in Boston. Wright's patient was an Italian woman who had resided in the United States for several years, but the disease had been noticed less than a year before she came under his care. The clinical manifestations of the infection and the pathological alterations in the specimen taken from this patient, as they are described by Wright, are very

[18] *Ann. de dermat.* (1892), May.

[19] *Am. Practitioner* (1876), 577.

[20] *Trans. Ass. Am. Physicians* (1895), 10, 92, and *Montreal Med. Journ.* (1905–6), 24, 485.

[21] *Am. J. Med. Sc.* (1899), N. S. 188, 393.

[22] *J. Exp. Med.* (1898), 3, 421.

similar to those which have been given by other authors. The description of the granules is very clear and Wright states that when bleached and softened by solutions of sodium hypochlorite or of potassium hydroxide, they are "found to consist of a mass of fungus structures, together with more or less brown, pigmented imbedding or investing material, as described and figured by Boyce and Surveyor." No spores were observed and the test for hæmin in the granules was negative. The latter were deeply stained by the basic aniline dyes, but not by hæmatoxylin. "The Gram stain and the Weigert fibrin stain, or modifications of them, stain the substance of the granules to a variable extent depending on the amount of the decolorizing agents applied." Wright cultivated a "*Hyphomycete*" from approximately twenty-five of sixty-five of the black granules. *Stapylococcus albus* also grew in most of his cultures. The growth developed from the granule used in inoculation and appeared after an interval of four or five days or even later. Growth occurred as follows in most of the media used:

Potato.—A dense, widely spreading, coherent layer of velvety surface; pale brown in the central portion and white at the edges. Small droplets of dark, coffee-colored fluid develop on the surface of the cultures. The medium becomes dark brown and very moist.

Bouillon.—Growth proceeds from the inoculated material in fine, radiating filaments and produces a puffball-like appearance, and eventually the whole fluid is filled up with radiating mycelia, the fluid becomes a deep coffee-brown color and a mycelium layer develops on the surface.

Potato infusion (20 grams boiled in water, with a finished filtered product of 1,000 cubic centimeters and not neutralized).—Growth is much the same as in bouillon, but no surface layer appears and in old cultures there are found numerous, black granules about 1 millimeter or less in diameter in the midst of the mycelium.

"These granules consist of closely packed, spherical or polyhedral cells, together with some short, thick, segmented hyphæ. The walls of these cells have a black appearance and masses of them are black and opaque under the microscope. Wright considered these granules to be "masses of interlacing hyphæ whose segments have been much shortened, and widened and otherwise changed." They were examined by W. G. Farlow and pronounced to be 'Sclerotia.'

Agar (plain and glucose).—"Growth appears as a mesh work of widely spreading filaments, of grayish color, on the surface of the medium." "Sclerotia" develop in old cultures, and in slant cultures growth only takes place on the surface.

Morphologically the *Hyphomycete* consists of long, branching hyphæ from 3 to 8 μ in diameter. Young forms show delicate transverse septa and older ones swellings at the points of branching and the hyphæ may appear as a string of oval-ended, plump segments. The filaments have a definite wall and branching occurs by lateral budding. No spore-bearing organs were observed.

Animal inoculations.—"No results were obtained from the inoculation of animals with the original granules or with cultures."

Histology.—"The tissues composing the nodules consist essentially of a formation of more or less atypical connective tissue in various stages of development in which foci of suppuration are present in association with granules.

"Some of the granules lie in small cavities containing polynuclear leucocytes, loose epithelial cells and cellular detritus. These cavities may be lined either by a wall of vascular granulation tissue or by massess of epithelioid cells, together with multinucleated giant cells. Other granules are closely invested by a zone of epithelioid and giant cells, and outside of this there may be an infiltration with lymphoid and plasma cells. The nodule thus formed about the granule resembles very closely a tubercle in structure. The giant cells are often of large size and may have a peripheral arrangement of their nuclei. They are a very prominent element in the lesions.

"The primary effect produced by the parasites upon the tissues seems to be the development of nodules of epithelioid cells and of giant cells from the tissues immediately about them. Later, suppurative processes occur in the nodules and abscesses are formed, which in the tissue give rise to the development of granulation and connective tissue in large amount."

Unna and Delbanco's (1900) discussion of the subject is mentioned by MacLeod. Brumpt (1901) reported a case of the black variety of mycetoma from Suakim, Sudan. He gives the usual description of the granules. The grains are composed of an enveloping membrane made up of mycelial filaments. The inner portion is very friable and easily crushed. Cultures in hay infusion and bouillon gave negative results. Fourlerton and Jones (1902) published an exhaustive article dealing with the pathogenesis of the *Streptothrix* group in general; the authors in discussing *Mycetoma* consider *Streptothrix maduræ* Vincent as the cause of the ochroid variety, and J. H. Wright's *Streptothrix* as that of the black type.

Laveran[23] studied an anatomical specimen of the black variety sent to him by Bouffard. He found and described a *Streptothrix* in the granules and named this organ *S. mycetomi*. The author does not report any experiments in the cultivation of the fungus.

Madden[24] published two cases of the pink variety of mycetoma occurring in the Sudan. In one of the patients the primary lesion was in the thigh, and later the disease involved the abdominal wall. Pinoy succeeded in one month in cultivating a *Streptothrix* in anaërobic, sweetened bouillon. With these cultures he was able to infect a pigeon's foot.

Scheube (1903) discusses the question of the identity of actinomycosis and Madura foot as follows:

"I need only recall the different size and coloring of the fungoid masses, in the two diseases, their different localization, and the difference of their course, which in Madura foot is more benign and more chronic than in actinomycosis. The pronounced tendency also for actinomycosis to spread to other near or distant parts of the body, to attack the internal organs, and the transmissibility of actinomycosis to healthy persons, are qualities which, at least according to our present knowledge, are not possessed by Madura foot. Moreover, the *hyphomycetes* of Madura foot are more delicate, and stain remarkable well with hæmatoxylin, showing the prisms and columns described above as staining with difficulty. Actinomycosis, on the other hand, is not stainable with hæmatoxylin and develops clubs and knobs which are difficult to stain."

Manson (1906) in discussing *S. maduræ* Vincent considers that "though closely allied to the better known fungus (*Actinomyces*), apparently it is not specifically identical with it."

Nicolle and Brunswic-LeBihan[25] report mycetoma in an Arabian woman. The original lesion was produced by a barley spore introduced one year before the mycetoma was established, and Nicolle and Pinoy[26] report a case of the ochroid variety of the disease in an Arab. They described and cultivated a *Streptothrix* from the lesions. The organism was grown upon sweetened hay infusion and developed on the surface of the medium as a light gray superstratum upon a brownish-red membrane. The authors consider carrots to be the best medium for rapid development of the organism, and growth occurs best at 37°, appearing as a white scum which rapidly becomes yellowish. Small spots of various colors also develop, according to the acidity of the media, the colors varying from grayish to brick red. Roux tubes in which were placed reeds and water, sterilized, constitute a favorable culture medium. In less than forty-eight hours a glaucescent growth covers the surfaces of the reeds.

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[23] *Bul. Acad. de Med.* (1902), (3), 47, 773.

[24] *J. Trop. Med.* (1902), 5, 243.

[25] *Bul. Acad. de Med.* (1906), (3), 55, 132.

[26] *Arch. d. Parasit.* (1906), 10, 437.

Potato.—A white growth develops in forty-eight hours, this increases up to eight days, becoming brownish. The medium becomes dark.

Gelatin.—On this medium the growth is very feeble and no liquefaction occurs.

Agar and Sabourand's gelose.—The growth consists of a brownish, folded membrane frosted with white, slowly becoming yellow.

Animal experiments.—Subcutaneous inoculation of monkeys and rabbits gave negative results. Subcutaneous inoculation in the foot of a rat produced small granulations which contained mycelial filaments; these granulations were gradually reabsorbed. Intravenous inoculations in rabbits were without result. The author discusses the question of the etiology of the disease and the identification of the fungi.

E. Brumpt[27] has written a critical review of the entire subject with particular reference to the parasites. He divides the fungi into two genera and several species, gives the generic and specific diagnosis of each, and places the previously described parasites in what he considers their proper position. He considers mycetoma to be a clinical type of disease which may be caused by any one of the several species of *Streptothrix* which have been described by various authors. The work is very exhaustive and the plates are excellent, but after close study it seems to us that his classification has been made upon insufficient data.

Foulerton (1907) in his recent and complete study of the pathology of *Streptothrix* infections in general, divides the group into several classes according to the lesions produced, the cultural characteristics, and so on; in one of these groups he includes *S. maduræ* Vincent and the other allied organisms which from time to time have been published as the etiologic agents in Madura foot. The author was unable to demonstrate acid-fast properties in cultures of *S. maduræ* with which he worked. In considering the acid-fast properties of *Streptothrix* in general, the author states that the number of these organisms is still too small to justify fully definite conclusions, and in addition to *B. tuberculosis* and other well known bacilli of the same type, he mentions *S. eppingeri, S. nocordii, S. capræ* as well as Sabrazis' and Rivère's organisms as having a similar acid-fast character. As our own *Streptothrix* is of this class of acid-fast organisms, a comparative study of the group is interesting and will be taken up more in detail in a subsequent paper.

Wright[28] in Osler's Modern Medicine discusses the question of the existence of two varieties of the disease. This author states that "at the present time the ochroid or pale form of mycetoma must be regarded as actinomycosis of the part." However, in the melanoid variety he recognizes the specific fungoid nature of the affection, although, as he himself states, the etiology of the disease remains to be proved by animal experimentation. Wright[29] was probably the first one to cultivate the fungus of the black variety. Its cultural characteristics have been given and are shown in the parallel column of Table II facing page 500.

[27] *Arch. d. parisit* (1906), **10**, 489.
[28] Osler's Modern Medicine (1907), **1**, 344.
[29] Loc. cit.

A very recent article by MacLeod[30] discusses the etiology of the disease in the following manner: "It is caused by the presence in the skin and underlying tissues of a *Streptothrix* closely allied to, but differing from, actinomycosis. The name *Streptothrix maduræ* has been given it by Vincent. This fungus forms the 'fish roe' like granules of the white variety, which is the common form of the disease." The author in describing the nature of the black variety states that there is a considerable variance of opinion, but he does not adhere to the belief that its fungus is a degenerative condition of the infection produced by the white type, but rather that it is a different species, as is claimed by several writers. MacLeod also recognized a red variety of the mycetoma in addition to the ochroid and melanoid types. In considering the differential diagnosis of the disease from actinomycosis the author states: "Actinomycosis is a disease which is transmitted from animals to man, and can be inoculated in animals; it occurs in temperate latitudes, often runs a rapid course, affects internal organs and mucous membranes, and has yellow granules in its discharges; Madura foot is confined to man, has not been successfully inoculated in lower animals, occurs in the tropics, runs a slow course, does not become generalized, and presents granules of various colors."

Caminiti[31] (1907) reports a new *Streptothrix* and discusses very fully the whole group of *Streptothrix;* however, but little attention is given to mycetoma.

DISCUSSIONS AND CONCLUSIONS.

The study of our case of Madura foot, a report of which is given in the first part of this paper, after a careful review of the literature, establishes for the first time definitely so far as we have been able to determine, the etiology of mycetoma according to the usual requirements of investigators, including the transmission of the disease by animal experiment. The causative organism we have determined to be a *Streptothrix,* and as it apparently differs from previously described fungi of the same genus we have named it *Streptothrix freeri.* We believe this investigation fully establishes the etiologic importance of *S. freeri* in one case of the pale or ochroid variety of Madura foot, but nevertheless we are of the opinion that it does not finally settle the entire question of the etiology of the disease. Some of the most important questions open for discussion are as follows:

1. Is *S. freeri* sufficiently different from previously described organisms to entitle it to be classified as a new species?
2. Is mycetoma a distinct disease etiologically, or is it one type of manifestation of more than one species of *Streptothrix* infection?
3. Are the "ochroid" and "melanoid" varieties different stages of a single etiologic entity, or are they due to different species of *Streptothrix*?
4. What is the relation of mycetoma to actinomycosis?

In spite of all of the admirable older investigations on the subject and giving all due credit to the writers of former contributions, there are but two articles dealing with the etiology of the disease which are sufficiently clear and which follow required methods closely enough to be of value in a comparative study. These are Vincent's report on *S. maduræ* as the cause of the infection in the ochroid variety, and Wright's article reporting a *Hyphomycete* as the etiologic factor in the melanoid type of the disease. The work of both these authors seems to have been carefully carried out and their conclusions appear to be sound, but in neither investigation did the authors establish the etiologic relation of their organisms to Madura foot by experiments on animals. However, leaving this omission out of consideration and studying the cultural characteristics of their fungi alone, the evidence seems to be conclusive that *S. freeri* differs from both of their organisms. This is not only so, but when we compare its characteristics with the description of other species of pathogenic *Streptothrix* which have been cultivated by others, we find our organism clearly to be distinct. The table opposite gives a comparative summary of the characteristics of six of these *Streptothrices*, and it includes those of *S. actinomyces*.

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[30] Albutts' System of Medicine (1907), **2,** 754.

[31] *Centrbl. f. Bakteriol.* Orig. (1907), **44,** 193.

There can be no reasonable doubt but that all types of mycetoma are due to *Streptothrix* infections, but whether all the forms are caused by an infection with a uniform organism or whether more than one species plays a part in the disease, can not now positively be stated. However, it is very probable that Madura foot may be produced by any one of several species of *Steptothrix*, and that lesions of etiology identical with the ones occurring in the foot may be produced in various parts of the body. Such infections are now occasionally recognized. It seems likely that some of these lesions, located in places other than the foot, are not considered to be mycetoma, more because of their location than because of any specific differences in the parasites. However, if the methods employed by observers who have reported mycetoma in parts of the body other than the foot are examined, it is certain that the diagnoses of these infections were based upon morphologic considerations of the *Streptothrix* as a whole, and therefore were not conclusive as to the determination of *species*.

S. freeri is as pathogenic for monkeys, when it is inoculated in the deep tissues in other parts of the body, as it is when injected into the foot, where it produces typical mycetoma clinically and pathologically. It seems probable that mycetoma most often occurs in the foot because of the accidents to which that member is exposed and in this connection it may be stated that the right foot is more frequently affected than the left one. If the general infections produced in animals by *S. freeri* prove to be experimentally practical with other types of the disease, then Madura foot becomes a variety of streptothricosis differing from other types more in anatomical position than in specific etiologic distinctions.

The review of the literature which has been given shows that there is much difference of opinion in regard to the etiology of Madura foot.

TABLE II.—*Cultural characteristics of various types of Streptothrix and of Actinomyces.*

	Streptothrix of Nocard according to the Fullerton.	Streptothrix of Eppinger according to Fullerton.	Streptothrix of Vincent according to Vincent and Fullerton.	Streptothrix of Wright according to Wright.	Actinomyces according to Wright.	Streptothrix freeri according to Musgrave and Clegg.
Source	Cattle in Guadalope	Man; brain abscess	Man; foot	Man; foot	Man and lower animals	Man; foot.
Morphology				Long branching hyphæ from 3 to 8 μ in diameter. In young forms delicate transverse septa are present. In older forms there may be swelling at these points and the hyphæ may appear as a string of oval-ended, plump segments. The filaments have a definite wall, and in their interior granules or pale areas may be seen. The branching occurs by the formation of lateral outgrowths or buds. No spore-bearing organisms were observed.	Varies in the different media. Sometimes forms clubs	Long branching filaments, 2 to 7 μ in diameter. Transverse segments are shown of various lengths from coccoid to 10 μ in length. Branching occurs as lateral hyphæ developing from the segments. The filaments have a definite wall. Spores have not been observed.
Staining characteristics	Cultures from 9 days to 3 months old stained by Ziehl-Neelson method showed many acid-fast portions and these were also alcohol-fast.	Cultures from 2 to 3 months old stained by Ziehl-Neelson method showed portions which are acid-fast but not alcohol-fast.	Cultures up to 14 weeks old stained by Ziehl-Neelson method showed no acid-fast portions.	Gram's stain and Weigert's fibrin stain, or modifications of them, stain the substance of the granules to a variable extent, depending upon the amount of decolorizing agent applied. The marginal portion of the granules hold the stain longest. In only a very few instances could the hyphæ be differentially stained by these methods.	All strains of the micro-organism stain well by Gram's method with the exception of one. Smear preparation from cultures of ten cases were not found acid-fast toward Gabbet's decolorizing solution after staining with carbol fuchsin.	Smear preparation from lesions and cultures from 5 days' to 2 months' old stained by Ziehl-Neelson's method showed many acid-fast portions. These were also alcohol-fast. Stain well by Gram's method.
Growth on gelatin, 22° C.	Aërobic. Streak cultures: Growth appears after 4 days as a fine, white opacity with uneven surface. Stab cultures: Slightly raised knob at top of gelatin and a fine, filmy growth along needle track. No liquefaction or pigmentation of medium.	Aërobic. Streak cultures: Growth appears after 6 days as a slightly raised, wrinkled layer with a deep orange center, and a paler often thinned out periphery. No liquefaction or pigmentation of medium.	Aërobic. Growth appears after 8 to 21 days, always scanty, opaque, whitish, raised, more or less discrete colonies. No liquefaction or pigmentation of medium. Stab cultures: Series of superimposed flattened globular opaque, whitish, colonies occur along needle track.	Aërobic	Anaërobic preferences	Obligative aërobic. Growth appears after 3 days as dry, opaque, discrete colonies. No liquefaction. After 3 months medium becomes slightly darkened. Stab cultures: No growth along needle tract.
Maltose agar	Appears after 48 hours as a pale, straw-colored growth, with dull, flattened, granular, surface; no pigmentation of medium.	Growth appears after the fifth day as a slightly raised yellowish mass with a pale, flat, polished periphery; later the growth assumes a deeper yellow to a burnt-ocher color. No pigmentation of medium.	Growth appears after 8 days as small, discrete, yellowish-white colonies with waxy, wrinkled surface; later often developing pink pigment.			Appears after 3 days as a dry, smooth, opaque and slightly raised growth; at first a porcelain white, which later develops a delicate pink color. No pigmentation of medium.
Peptone broth	Growth appears after 24 hours as a grayish, flocculent mass at the bottom of the tube. No pigmentation of medium.	Growth appears after 24 hours as a small, white granules, forming a pellicle and adhering to sides of tube; some portions fall to the bottom. No pigmentation of medium.	Growth appears as opaque, white, globular, more or less cohering colonies at bottom of tube. Surface growth rare. No pigmentation of medium.	Growth always proceeds from the planted material, whether grains or fragments of mycelium in the form of fine, radiating filaments and soon produces a puffball appearance. Eventually the whole area of fluid is filled with radiating mycelium and a definite mycelial layer is formed on the surface. The medium acquires a coffee-brown color.	Growth occurs in bouillon in the form of solid, whitish masses at the bottom of the tube; there is never growth at the surface. When first isolated from the lesions these masses of growth appear in the form of nodular, irregular, spherical structures, often coherent to one another and forming mulberry-like masses. Under continued cultivation most of the strains of the microörganism grow in the form of flaky, friable, amorphous masses, which in some instances after some days in the incubator became transformed into stringy, viscid material. One strain after months of cultivation still retained its mulberry like character. Medium not clouded.	Growth occurs after 3 days as flat particles on the surface of the medium. These produce in time a delicate, pink color, and on shaking the tube adhere to its sides. As the growth proceeds a granular mass collects in the bottom of the tube, this mass is more or less coherent. Medium does not become cloudy but in time becomes darkened.
Alkaline litmus milk	Growth occurs, but reaction and appearance of medium not obviously altered after 20 days' incubation.	After 3 days the medium turns pink; by the eighth day the color is discharged and milk has become alkaline, but even after a month's incubation the medium does not clear.	Growth occurs. No obvious change happens in the milk until after 20 days' incubation; the medium then becomes pink and after 4 weeks begins to clear.		Tubes were inoculated with fourteen strains and maintained under both anaërobic and aërobic conditions. Some slight or doubtful growth was abserved on six.	Growth appears after 3 days on surface of medium as flat, dry-appearing particles. These later become confluent, forming a heaped-up, pinkish-yellow mass. No reaction occurs in medium until after second week when the color gradually fades but does not become red. This decolorization begins at the bottom of the tube. Milk is not coagulated.
Potato	Appears after 48 hours as a buff-yellow granular growth. No pigmentation or erosion of medium.	Growth appears after 48 hours as a granular layer, at first white, becoming yellow to brick-red. This is later covered by a fine, white, powdery efflorescence, culture resembling a sugar almond.	Growth appears after 72 hours as a heaped-up, irregular mass of a creamy-yellowish-white color, often showing patches of pink coloration. No pigmentation or erosion of medium.	It forms a dense, widely spreading, coherent membrane or layer of velvety surface, pale brown in color throughout its central portion and white at its peripheral portion. A marked feature is the appearance of small, spherical, globules or droplets of dark coffee-colored fluid on the surface of this layer. The potato takes a dark brown color and becomes moist.	Fourteen strains were planted and all except two were observed under both anaërobic and aërobic conditions; in no instance was anything like a luxurient growth obtained.	Growth appears as small, pinkish colonies, later raised and becoming confluent; after a few days the growth assumes a heaped-up appearance resembling a mass of yellow curls. Medium becomes moist and dark.
Potato infusion				In this the growth proceeds as in bouillon except that no surface layer is formed. A peculiarity noted in old cultures is the appearance of numerous, black granules about 1 millimeter in diameter in the midst of the mycelium.	Cultures of five strains were placed under anaërobic conditions. In no instance was evidence of growth obtained except in one tube, in which there might have been a slight growth.	Growth similar to bouillon.
Löffler's blood serum and inspissated horse serum.	Very slow growth. No liquefaction of medium	Growth appears after 48 hours. No liquefaction or pigmentation of medium.	Growth slow and scanty. No liquefaction or pigmentation of medium.		In some instances a very slight growth appeared, in others none.	Growth appears after 3 days smooth and of a delicate pink color. Growth does not change character of medium after 3 weeks' incubation.
Glucose agar				The growth appears as a mesh work of widely spreading filaments of a grayish color on the surface of the medium. In old cultures black sclerotia are found as in potato infusion. In stab cultures growth appears only at the surface.	do	Growth appears after 3 days, later becomes luxuriant with a heaped-up and wrinkled appearance. The center assumes a pinkish-yellow color, with a gradual elimination to a delicate pink and to a white periphery. Stab cultures show only surface growth with same pigment characteristics.
Glycerin agar					do	Grows well, appearing after 3 days as a raised, heaped-up, moist growth; produces a yellow pigment in center, with a pale periphery.
Diastatic action	None manifested during 14 days' incubation in starch, peptone and broth.	None manifested during 14 days' incubation in starch, and peptone broth.	None manifested during 14 days' incubation in starch, peptone and broth.			
Resistance to heat, drying, relative growth to temperature, thermal death point.	Sporulating cultures resist an exposure to 45° C. for 30 minutes, but are killed at an exposure to 60° C. for the same period. (Cultures resist exposure to 65° for 15 minutes but are killed by exposure to 70° C. for 10 minutes.—(Nocard.)	Sporulating cultures resist an exposure to 60° C. for 30 minutes, but are killed at an exposure to 75° C. for the same period.	Sporulating cultures resist an exposure to 45° C. for 30 minutes, but are killed at an exposure to 60° C. for the same period.		Growth observed practically only at 37° C. Resistance to drying showed that the microörganism survived longer on plain surfaces than in culture media, 50 days compared with 6 to 10 days according to strain used. Thermal death point 60° to 64° C. for 10 to 11 minutes.	Is killed at an exposure to 70° C. for 15 minutes.
Pathogenicity for lower animals	Rabbits, dogs, cats, horses, and oxen are not affected by intraperitoneal or intravenous injections. In guinea pigs intraperitoneal or intravenous injections cause constantly within 20 days a miliary tuberculosis similar to that produced by bacillus tuberculosis.	Not pathogenic for white mice. Three rabbits inoculated subcutaneously recovered after developing temporary local swellings. One rabbit inoculated in anterior chamber of eye developed a nodule on the iris but no general infection. One rabbit inoculated intravenously and another in the kidney died on the fifth and seventh days respectively. Two guinea pigs inoculated intraperitoneally died on the eighteenth and twenty-fourth day.	The inoculation of cats, rabbits, guinea pigs, and mice produced nothing more than a small nodule at the site of injection and these disappeared (Vincent). Nocard carried out intraperitoneal, intravenous and subcutaneous inoculations of dogs, sheep, rabbits, guinea pigs, fowls, and pigeons; his results were negative in every case.	No results were obtained from the inoculation of animals with the original granule or with cultures.	Guinea pigs and rabbits were mostly used in these inoculations. The results were not constant; with some stains typical lesions were produced which were anatomically and histologically identical with those of actinomycosis.	Intraperitoneal inoculations of guinea pigs, monkeys, and dogs with material from lesions and from cultures in nearly every case produced processes resulting in death after two weeks. Rabbits and pigeons were not affected other than by the production of small nodules at site of inoculations in subcutaneous injection; these soon underwent resolution and healed. No progressive lesions were produced by subcutaneous inoculations. In monkeys inoculated in the foot with cultures extensive lesions were produced anatomically resembling Madura foot. Pure cultures were obtained in every instance from experimental lesions.

The only important data tending to show that different types of the disease exist are found in the varying color of the granules from the different varieties and the results of Wright's cultivation experiments. While Wright could not establish the etiologic importance of his organism, it is probable that it was the cause of the disease in his case; this being true, the etiologic identity of the two varieties must be questioned until further experimental work is done with both Wright's and our own organisms.

One of our experiments on monkeys is suggestive. A fair number of small, black granules were produced in the tissues of monkey number 3267 inoculated subcutaneously, and a less decided, but positive, variation in the color of the granules has been noticed in other inoculated animals.

The statement which has often been repeated, that the ochroid variety of mycetoma is actinomycosis, is not supported by the weight of evidence in the literature, and it is positively disproved by our work.

There is no doubt but that actinomycosis has been mistaken for Madura foot, both of the ochroid and black variety, in some of the reported cases, but it is equally certain that *Actinomyces hominis* is a different species of *Streptothrix* from those producing mycetoma. Furthermore, the two diseases show decided differences in clinical manifestations and in the pathologic findings.

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Madura foot (Mycetoma). *In:* Osler's Modern Medicine (1907), **1**, 344–346.

ILLUSTRATIONS.

PLATE I.

FIG. 1. Madura foot, mycetoma.
2. Monkey's foot after ten days. Inoculated with cultures from the lesions of foot shown in Plate I, fig. 1.
3. Monkey's foot after fifteen days. Inoculated with scrapings from the lesions of foot shown in Plate I, fig. 1.

PLATE II.

FIG. 1. Section from the foot of the original case; stained preparation. 1 × 75.
2. Section from the foot of the original case; stained preparation. 1 × 100.
3. Section from abdominal tumor of dog number 3390; stained with Sterling's gentian-violet iodine solution (1–2–200) decolorized with aniline oil. 1 × 390.

PLATE III.

FIG. 1. Fresh smear from the lesions of the original case. 1 × 850.
2. Fresh smear from a thirty days' culture on glycerin-agar. 1 × 850.

PLATE IV.

FIG. 1. Drawing of a ten days' culture of *Streptothrix freeri* on glycerin-agar.
2. Potato culture of *Streptothrix freeri* showing ten days' growth.

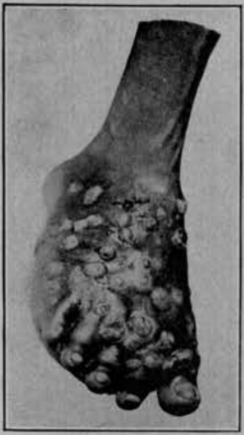


FIG. 1.

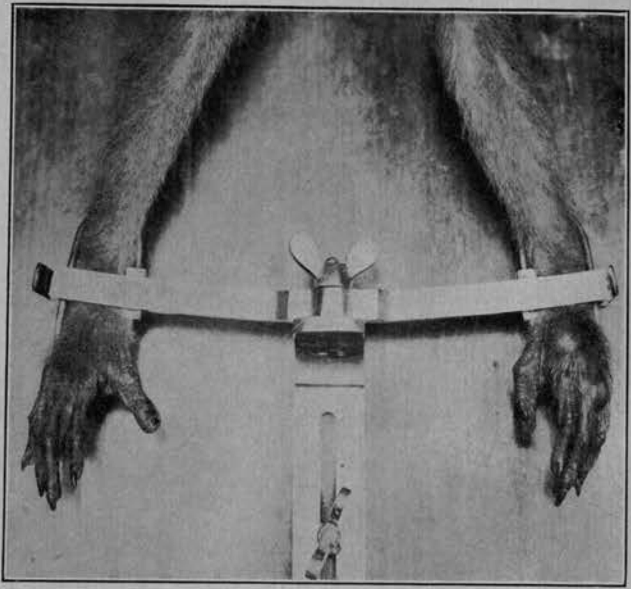


FIG. 2.

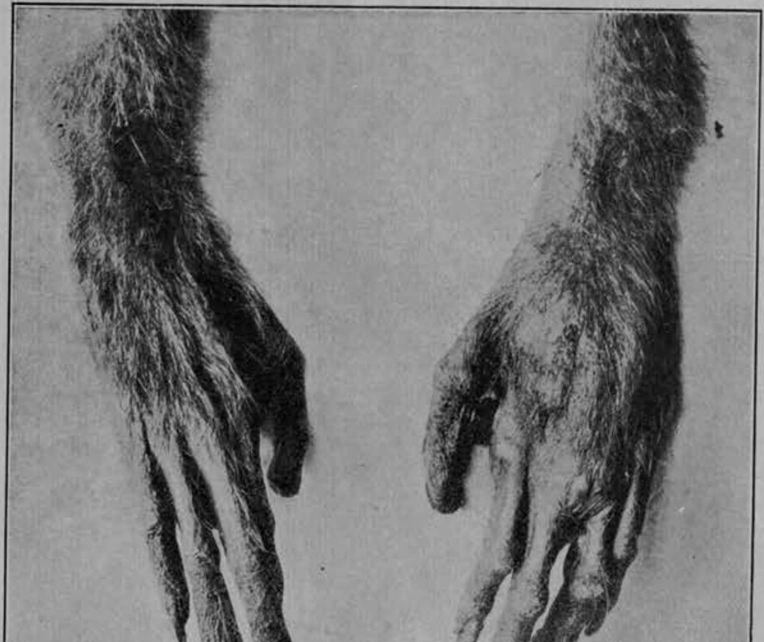
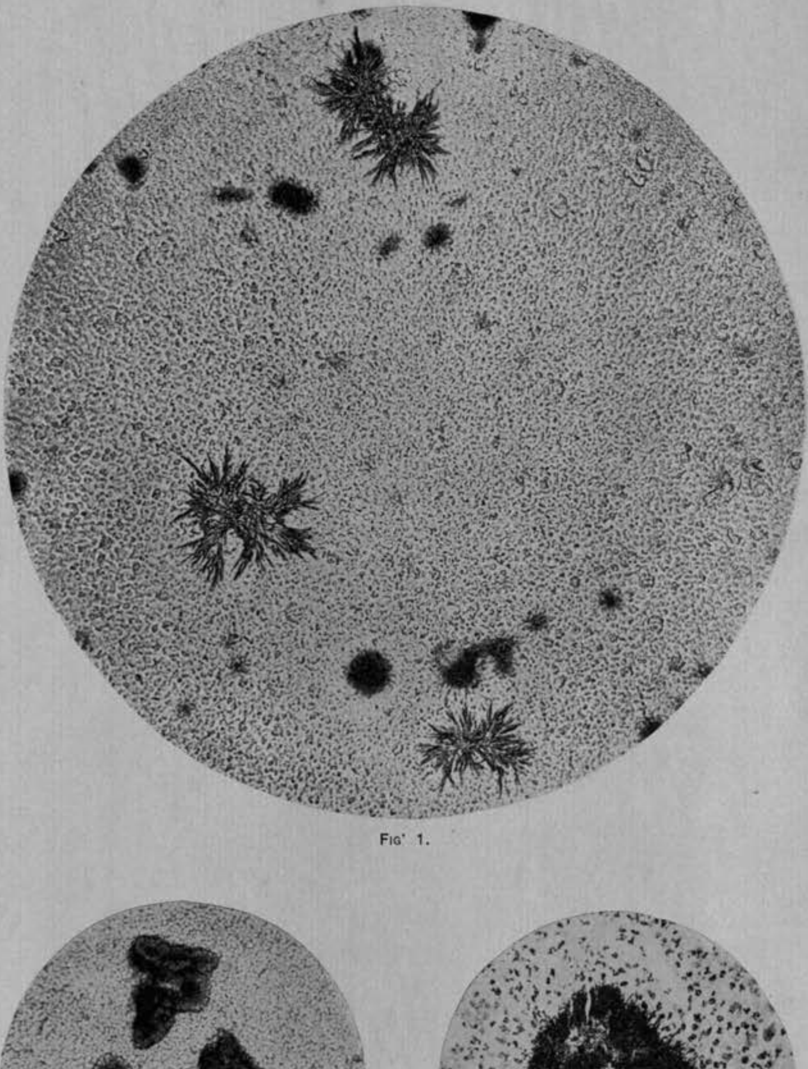
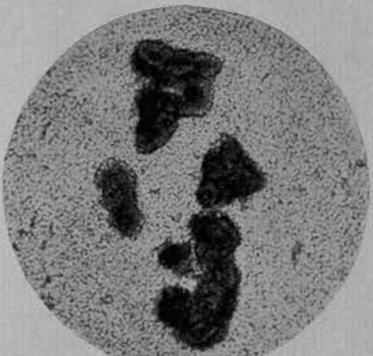


FIG. 3.

PLATE I.



FIG' 1.



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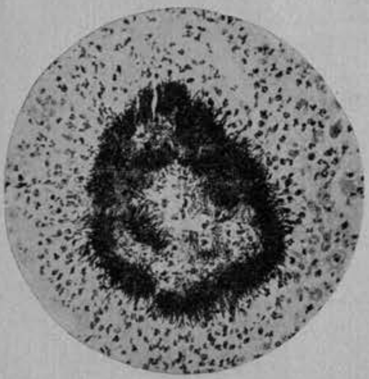

FIG. 3.

PLATE II.

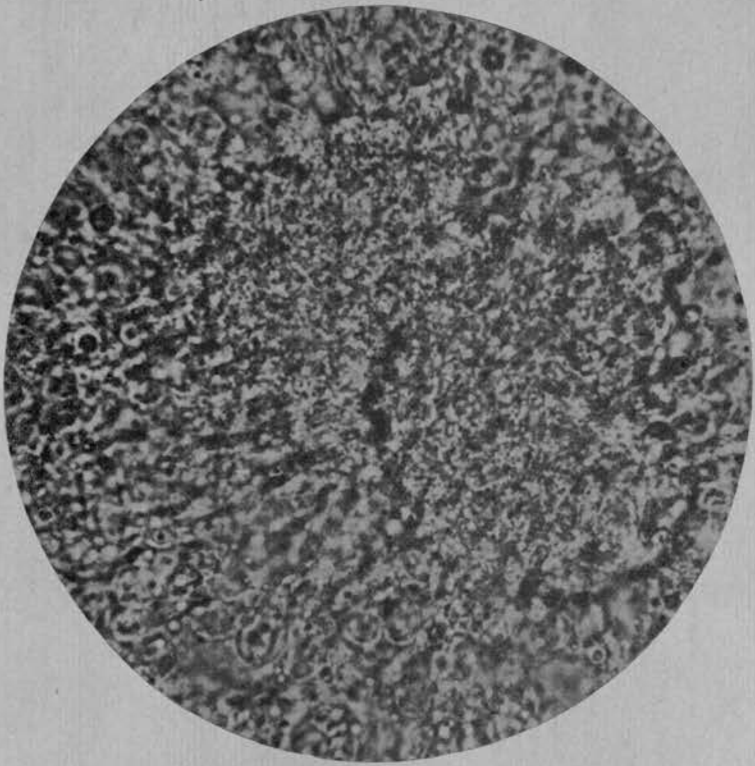

FIG. 1.

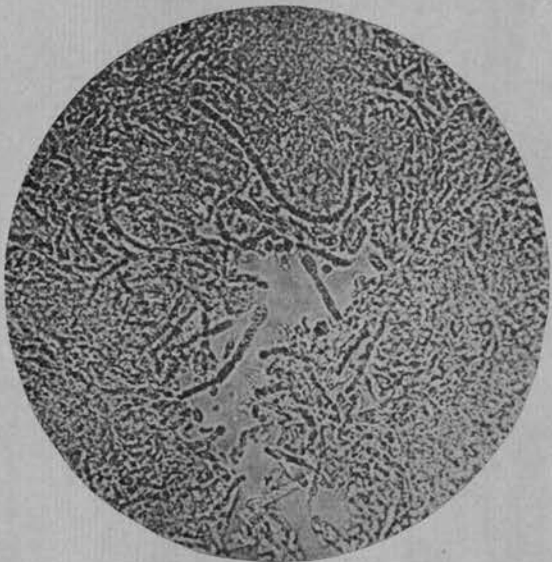

FIG. 2.

PLATE III.

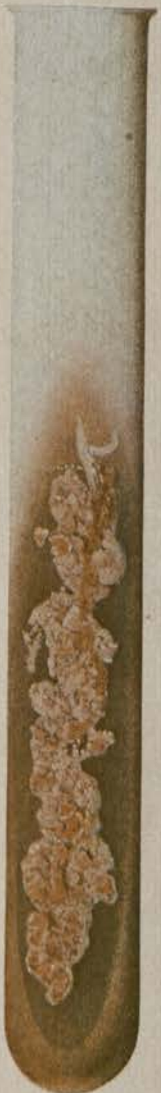

FIG. 1.

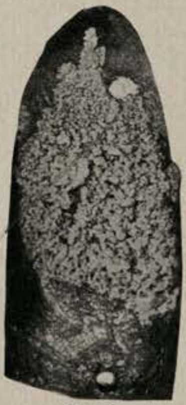

FIG. 2.

PLATE IV.

EXPERIMENTS IN MALARIAL TRANSMISSION BY MEANS OF MYZOMYIA LUDLOWII THEOB.

By Charles S. Banks.

(*From the Entomological section, Biological Laboratory, Bureau of Science, Manila, P. I.*)

1. Introduction.
2. Breeding Places of the Mosquitoes.
3. Life History of Myzomyia ludlowii Theob.
4. Mosquitoes and Malarial Parasites.

INTRODUCTION.

Physicians in the Philippine Islands have for several years been desirous of determining with greater certainty the exact species of *Anophelinæ* responsible for the transmission of malaria in this region, although as far as I am aware no definite experiments looking to this end have ever been carried on.

In the year 1906 a very severe epidemic of æstivo-autummal fever, resulting in several deaths both among the American and Filipino workmen, broke out in a camp some 6 kilometers from Manila on the line of the new waterworks excavations. I went to the scene of the trouble to study the features of the infection, but not until some time after it had been decided to abandon this camp and all the workmen, including also the families of the Filipinos, had moved away. A very brief visit of two or three hours, made at a time previous to the breaking up of the camp, enabled me to determine the actual state of existence of these people and to find out that practically none of the Filipinos and very few of the Americans used mosquito nets. A great many specimens of *Nyssorhynchus barbirostris* Theob., were found among clothing in the huts of the workmen.

A reconnaissance of the immediate vicinity of the camp at the time of the second visit revealed the abundant breeding places of the species of mosquito just referred to, but no further attempts were made definitely to fix the blame of malarial transmission upon the species found.

In December, 1906, word came from the United States naval station at Olongapo, Zambales, that malaria was very prevalent among the large body of marines in barracks at that place and that it was of a very pernicious type. It had been noted for some time previously that companies going for target practice to the marine rifle range at Maquinaya, some 5 kilometers north of Olongapo on the east shore of Subig Bay, invariably returned to Olongapo with a large percentage of their personnel infected with malaria, in one instance as many as 85 per cent having the fever.

It was not possible, owing to pressure of other work in Manila, for me to reach Olongapo before the 1st of April, so that upon my arrival I found the disease to have diminished markedly, although cases were by no means of rare occurrence.

GEOGRAPHY AND TOPOGRAPHY.

The naval station of Olongapo is situated at the southeast end of the small island of that name, lying in the eastern part of Subig Bay and separated from the mainland of Luzon by the Kalaklan River. It is really one of a series of deltas formed at the mouths of the Kalaklan, Santa Rita and Biniktigan Rivers. The region roundabout is hilly, often to the coast, the main axes of the hills running at an angle with the coast line and having broad valleys between them, especially on the east shore of the bay. More or less sluggish streams flow through these valleys and upon reaching the coast they naturally spread out, forming areas of mangrove swamp in which a certain species of large crustacean has built up mounds of earth which in time, owing to vegetation and the further deposit of earth by the rivers, have united to form a more or less level tract having an elevation of from 3 to 10 decimeters above mean tide. Numerous channels are cut through these tracts of land at the time of floods, and when the tides are high the waters of these channels are continuously mixed with that from the sea. At no time is the water in or around these areas fresh, and where the tide recedes it leaves the landlocked pools to become more salty by evaporation. The shores for several kilometers around Olongapo in all directions are of this character, and this condition is only relieved by the terminal talus slopes of the surrounding hills.

Engineering operations carried on by the Navy with reference to harbor improvements at the station, have altered the course of the Biniktigan River while causing large bodies of water to become more landlocked than previously, with the result that now the area of stagnant or semi-stagnant water in the vicinity of the station is about doubled.

The same condition obtains at the Maquinaya rifle range, except that the swamps at this place lie farther landward, whereas the camp itself is located on a sandy beach, upon which fair sized trees of various species grow.

LOCATION AND BUILDINGS.

The navy-yard proper is located on a sand spit and is surrounded by a wall built in Spanish times; all the offices and the quarters of most of the naval and marine officers and the hospital are located within this wall. Outside, and along the west beach of the island are the other marine officers' quarters and eastward from these, at a distance of some 120 meters, are the five large buildings erected for barracks for the troops. Four of these barracks are within a few meters of the swamp regions. They are built on cement piers about 1 meter from the ground level and are well ventilated. Sewage is carried into Subig Bay by pipe lines and there is no stagnant water in the immediate vicinity. Each building is provided with a tank on the upper veranda into which water is pumped for bathing purposes and for flushing the closets. The ground area beneath and around the barracks is kept in a scrupulously clean condition, many of the posts and piers being frequently whitewashed and no weeds are allowed to grow. The parade ground at the west and south of the barracks is not yet finished and therefore it is cut in many places by trenches and ditches into which tide water finds its way and where *Culex microannulatus* Theob., and *Myzomyia ludlowii* Theob., breed in great numbers. Larvæ of both the above-mentioned species of mosquitoes were found at the Maquinaya rifle range, freely breeding in salt water less than 60 meters from the camp, which consisted of tents for both officers and men.

According to regulations, all men are obliged to sleep under mosquito nettings, but I am of the opinion that these do not completely serve their purpose in preventing the men from being bitten, for few sleep so quietly that at some time during the night their arms or legs do not come in contact with the sides of the narrow nets, thus offering a ready mark for mosquitoes.

PLAN OF WORK AT OLONGAPO.

It was decided that the first requisite was the discovery of all species of mosquitoes which might play an important rôle in the transmission of disease, as well as to study the conditions under which the insects breed most readily. A considerable amount of time was consequently spent in reconnaissance in the neighborhood both of Olongapo itself and the rifle range at Maquinaya. During this period mosquito larvæ were collected and bred, and breeding grounds mapped out. This work enabled me to narrow the species of *Anophelinæ* down to *Myzomyia ludlowii* Theob., as the most probable transmitter of malaria.

FACILITIES.

The breeding grounds of *Anophelinæ* were so near to the laboratory where my indoor investigations were carried on, that it was an easy matter to go from the outdoor cages to the workroom several times a day if necessary. At first, certain places were simply watched daily and the development of the larvæ and pupæ noted, but as work went on it was found desirable to isolate individuals and eggs in order to determine with exactness the various periods of growth. I took with me from Manila most of the portable apparatus necessary, but many features which needed to be constructed on the spot were furnished by the naval authorities, all of whom, without exception, did everything in their power to aid my work. I wish in this place to express my thanks for their assistance.

SUBSIDIARY INVESTIGATIONS.

On the 22d of April a battalion of marines, fully equipped for heavy marching, left Olongapo under the command of Maj. E. K. Cole, United States Marine Corps, for a military reconnaissance of the region around Mount Pinatubo to the north of Olongapo and distant some 48 kilometers. This expedition from the time of starting until the return to Olongapo occupied five and a half days, during which time the whole command slept at no one place for two consecutive nights. We left the seacoast at the town of Subig 12½ kilometers north of Olongapo at the head of Subig Bay and struck inland, there being a continuous and gradual rise in the land until the second day, when the country became strictly mountainous. The night camps were always pitched in the vicinity of running water, but in no case was stagnant or semistagnant water encountered during the trip. A few mosquito larvæ were found at Santa Fé in the river near the camp, but the specimens collected died before reaching maturity. However, they were not Anophelines.

On the last night of the expedition (April 26) at a small place south of Castillejos, Zambales, the sleepers were annoyed by considerable numbers of *Devoidya joloensis* Ludlow (*D. fusca* Theobald, var. *joloensis* Ludlow).[1] This species probably breeds in the rather sluggish river near this place, although a search did not reveal the larvæ at that time.

These mosquitoes were the only ones encountered on the trip and it is noteworthy that no single individual of the entire expedition, comprising some one hundred and twenty-five men, was attacked by malaria subsequent to his return to Olongapo, within the usual incubation period of the disease; in fact, a careful observation of the men of the command for two months after their return showed that, where there was any malaria, it was a recrudescence of the æstivo-autummal type and in individuals who had been suffering from the disease for some time before this trip.

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[1] Theobald says in *Mono. Culic.* (1907), 4, 165, that this variety merits specific rank and I have so indicated it.

THE BREEDING OF MOSQUITOES.

GENERAL CONSIDERATIONS.

Most of the species of mosquitoes breed in fresh water; in ponds, sluggish streams, the margins of lakes and swamps or in cisterns, wells, water tanks and all other receptacles for collecting water around houses, which are not covered by wire gauze or other mosquito-proof covering. Certain species breed only in open meadow or marsh waters, others only in woodland waters and again others only in receptacles in or near houses. Examples, respectively, of these three classes in the Philippines are *Nyssorhynchus barbirostris* Van der Wulp, *Desvoidya fusca* Theob., *Worcesteria grata* Banks, *Culex fatigans* Wied., and *Stegomyia persistans* Banks. The total number of known species of Culicidæ found to breed in salt or even brackish water is so very small that the finding of a true anopheline breeding under such conditions is remarkable.

Only the following species of *Culex* are known to multiply in salt or brackish waters in the United States: *C. solicitans* Walk., *C. salinarius* Coq., *C. tæniorhynchus* Wied., and *C. cantator* Coq. In Europe Bancroft and Ficalli record larvæ of *Culex salinus* Fic., from salt water, while Theobald adds *Culex marinus* Theob.

Three species of mosquitoes have been found breeding in salt water in the Philippine Islands; they are *Culex microannulatus* Theob., *Myzomyia ludlowii* Theob., and *Culex* sp. indet. In a former publication [2] I stated that *C. microannulatus* Theob., breeds in brackish water, but it has since been found in the same places as *M. ludlowii* Theob.; that is, in water which upon analysis yields a greater percentage of salt than that of Manila Bay. *Myzomyia ludlowii* Theobald was the species used in the transmission of malarial parasites in the series of experiments recorded in this paper. It is quite evident from collections made in the various parts of the Philippine Islands, that this species is quite generally distributed. It has been taken in Pampanga Province, in Jolo, Panay, Negros, Cebu, Manila, Cavite and Mindoro, so that its range is probably general in this Archipelago. It is always found in what we might term tidal backwaters, namely in swampy regions where the incoming tide floods considerable areas intersected by channels and dikes. In these places a certain amount of water is left by the outgoing tide, and as evaporation takes place, the remaining water becomes more salty until the next tide, when it is once more restored to nearly its ordinary specific gravity.

The amount of rain during the season when these mosquitoes are most abundant, namely from November to July, is not sufficient to render the water which remains in their breeding places sufficiently fresh to injure or destroy the plant life upon which the mosquitoes feed, or to be detrimental to the insects themselves; on the other hand it has been conclusively proved that they can not live in fresh water, nor in salt water which has been brought up to the point of saturation. Certain species of algæ are always found in salt water where *Myzomyia ludlowii* Theob., breeds. The commonest of these forms are *Enteromorpha* sp. indet. and *Chaetomorpha* sp. indet.[3] and the cells of these plants are found in the mosquitoes' stomachs. The insects are top feeders and make little or no use of the slimy algæ which grow upon the muddy bottoms of the pools or ditches in which they live, unless the water has so far receded as to bring these plants to within half a centimeter of the surface. Few other water plants are found in the breeding places of *Myzomyia ludlowii* Theob., but these, where they occur in small quantity, do not appear to affect the larvæ. The fact that none of the latter are found in water the surface of which is covered more or less completely with leaves of aquatic plants, goes to prove further that these insects in the larval and pupal stages thrive best under the almost uninterrupted glare of the sunlight, and that they retire to the shaded places only at the time of metamorphosis, when they remain below the surface for considerable periods before they came to the top to breathe.

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[2] *This Journal* (1906), **1**, 988.

In laboratory breeding experiments the plants in the water begin to die within three to five days, while the larvæ appear to feed in a desultory manner. The time for their natural transformation to the pupa comes and goes and they still remain as larvæ. The foulness of the water, due to organic decomposition, appears not to affect them, but on the other hand, the lack of proper food seems to cause them to remain in an indefinite larval stage, until after three weeks or more they gradually begin to die. Pupæ brought in and kept under similar conditions develop normally and the mosquitoes emerging from them appear not to have suffered from lack of sunshine in their previous stage. The conclusions are, therefore, that direct sunlight is absolutely necessary for the best development of this mosquito.

In order to obtain a sufficient number of individuals for experiments in biting, a large, white, gauze net was built over a pond the area of which was approximately 7 square meters. This net was high enough so that a man could enter by stooping down, and go from one part of the inclosure to the other on boards about 15 centimeters above the surface of the water. The earth banked up inside and outside the net held it in place and kept the mosquitoes from creeping out beneath the flaps; it also served as a resting place for the newly emerged adults. They were never found upon the net itself, but always upon the earth, and being so nearly of the same color it was not an easy matter to find them.

While there was no appreciable difference in the appearance or rate of development of the larvæ, the adult mosquitoes were of a slightly paler color when they were reared under nets and for some reason appeared to be less active or wary than those individuals encountered in the open.

Care was taken in constructing the net to exclude all small fish that might be enemies of the mosquito larvæ, so that when masses of algæ containing large numbers of mosquito eggs were placed in the water, the insects could develop without menace from this source. However, it is probable that a limited number of the larvæ and pupæ fell a prey to the tiny water beetles and their larvæ, which could not all be removed from the water owing to the fact that many of them burrowed in the mud when the pond was disturbed. One or two dragon-fly larvæ were left in the water and they reached maturity. They were seen to capture some of the hundreds of *Myzomyia ludlowii* Theob., and *Culex microannulatus* Theob., which emerged, but their quota must have been very insignificant, since they were small damsel-flies and not the larger and more voracious *Libellulinæ*.

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[3] I am indebted to Dr. W. R. Shaw, Philippine Normal School, for these identifications.

One of the most satisfactory methods of collecting the adult mosquitoes for experiment was to permit a large number of the larvæ to pupate under the net among the algæ and, within a day of the time for their emerging as adults, to collect as many as needed with a scoop net. They were then placed in a jar of salt water and covered; the next morning all of the adults would be found, they having emerged during the previous evening.

Many attempts were made to raise these mosquitoes in confinement, but I succeeded only after numerous trials. Under ordinary conditions evaporation is so rapid that water must be added each day; this so disturbs the larvæ that they do not thrive well, especially when an attempt is made thoroughly to mix the water at the time of its addition. Another detrimental feature is the amount of reflection of light from the sides of the vessel. Thermometric tests show that the air just above the water contained in a 10-liter porcelain evaporating dish is from 5° to 7° C. above that of the outside. In 20-liter cylindrical glass vessels of 35 to 40 centimeters height it is from 8° to 10° C. Direct sunlight does not detrimentally affect the larvæ and pupæ, but the adults die within fifteen to twenty minutes when so exposed, so that those emerging under such conditions in a covered vessel never reach the stage where they can fly. Those not killed directly by the sunlight become so thoroughly soaked by the moisture collecting on the sides of the vessel that they soon die. It is possible, by using a large porcelain evaporating dish and shading a portion of it with a black cloth under white, to keep the temperature down 2° or 3°, but even under such conditions great vigilance and a repeated change in the position of the vessel to a shady place for an hour or so and then back into the sunshine, is necessary.

LIFE HISTORY OF MYZOMYIA LUDLOWII THEOB.

The egg.—One and eight-tenths millimeters in length and 0.95 millimeter at its widest point including the air-cells, it being slightly wider than deep. The egg (Pl. I, figs. 1 and 2) is nearly jet black, the surface being covered with an iridescent, reticulated film which separates when the egg dries. It is blunt canoe-shaped, the lower surface strongly convex, the upper concave lengthwise. At each end is a small, round, lighter area having 7 to 8 tiny black dots within its circumference. A rim of air-cells extends around the entire upper part and at each side near the top and midway between the extremities occupying nearly one-half the length is an arrangement of air-cells which extends from the top halfway down the side of the egg (Pl. I, fig. 2). These cells run dorso-ventrally, each one widening downward.

The eggs are usually laid in the afternoon after 4 o'clock. When ovipositing the insect alights upon the water at a place where a mass of algæ exists. After walking around for a brief time, it finally extrudes the eggs one after another, at the rate of about one per second until five or six have been laid; it then walks a short distance away and then deposits a few more. It has thus far been impossible to determine the number laid by a single female, but counts of the eggs found in the ovaries and oviducts of new females show it to be seventy-five to one hundred and fifty, the average being about one hundred.

Larva (Pl. I, fig. 3).—The larvæ hatch in from thirty-six to sixty hours from the time of oviposition, the period of incubation depending upon the temperature.

The egg shell in hatching splits longitudinally on the upper surface, somewhat in the manner of the eggs of Muscidæ, the small piece breaking out and the shell afterward shriveling and rolling up. The young larvæ are pale gray, almost transparent and have a white spot on the anterior area of the thorax. The head is marked by a prominent, black band extending around the entire posterior margin and there are a few dark brown markings on its surface. The dark, triangular spots before the eyes persist throughout the larval period and are the beginnings of the adult compound eyes. This may easily be seen just before the last molt and when the pupa has formed within the larval skin.

The full grown larva (Pl. I, fig. 3) is greenish-gray, the abdomen is dorsally of this color, slightly darker near the median line. The region around the anterior dorsal and ventral portions of the thorax has a decidedly blue color subcutaneously. There is a small, transverse, black tergite between every two abdominal segments. These tergites appear at first sight to be the result of great transparency and the consequent visibility of the stomach and intestinal contents, but they are of course cutaneous. The ventral surface of the thorax and abdomen is pale bluish-green-gray.

The head is light buff, with the following dark brown markings above: The small, round eyes at the lateral prominences; a large subtriangular band, representing the compound eyes of the adult in front of each eye; a narrow band around the posterior rim of the head; a transverse, broken, wavy band five-eighths the distance across the top, before the eyes; a V-shaped line medially, opening anteriorly and connected posteriorly with the band on the posterior rim of the head; a small, triangular, median dot between the legs of the V; a small, round dot behind the eye, halfway from the posterior margin.

On the underside, the head is marked as follows: A large, brown patch on the ventro-lateral area posteriorly; a furcula-shaped mark, its apex at the mouth, extending posteriorly nearly to the margin; a small dot at the basal angles of the labium. The tips of the mandibles are black. (Pl. I, fig. 5.)

The labial plate is of peculiar structure, being bilaminate, the proximal lamina has 9 teeth, the external lamina 5. A thin, chitinous prolongation of the cusps extends into the head and serves as a brace. (Pl. II, fig. 4.)

The labium is composed of a movable portion which has 15 very stout, blunt, curved bristles along its margin and is covered with smaller, scale-like processes extending also to the clypeus. (Pl. II, fig. 2.)

Dorsally on each of the second to sixth abdominal segments, midway between the median line and the lateral angle, is a small tuft of stellate hairs, used for supporting the larva horizontally beneath the surface film. When it comes to the surface these hairs spread out, engaging the film. Each abdominal segment has laterally one or two pectinate bristles, diminishing in size toward the anal extremity.

Chætotaxy of the full grown larva.—In order to avoid confusion the arrangement of the hairs on one side of the median line is given.

Head (Pl. II, fig. 1).—At the base of the clypeus, projecting anteriad over and to end of the brush, is 1 straight, simple bristle, slightly to one side of the median line; postero-mediad to the base of the antenna 1 straight pectinate bristle and 2 more in a transverse line mediad to it and equidistant; exteriad and ventrad to the antenna, curving anteriad, is 1 pectinate bristle; ventrad to this is a group of 3 short, straight hairs of equal size projecting from the same point; posteriad to this another group of 3, antero-ventrad to the simple eye and slightly remote therefrom; anteriad to the median angle of the compound eye-spot is 1 simple bristle projecting at a right angle to the surface of the head; there are no hairs or bristles on the ventral surface. The maxillary palpus is armed with a compound, palmate-pectinate bristle at the outer side of the apex, before the articulation of the terminal joint. (Plate II, fig. 3.)

Thorax.—On the anterior face (Pl. III, fig. 2) laterad to the point of articulation of the head is a very small, bi- or tri-furcate bristle projecting anteriad; 2 long bristles in a transverse line on the anterior area of the dorsum, laterad of the median line; laterad of the exterior of these and distant from it three times its distance from the interior bristle, is a short, stout, pectinate bristle projecting anteriad; exteriad to this is another, more slender, nearly twice as long, projecting parallel with it, the latter followed laterad by a slender, simple bristle of its own length, projecting latero-anteriad; latero-ventrad to this is a pectinate bristle nearly twice its length, projecting latero-anteriad; ventrad of this is a curved, pectinate bristle projecting anteriad on the anterior face of the thorax; at the intersection of an imaginary transverse line through the middle of the thorax and one a little more than midway between the median line and the dorso-lateral margin, is a long, pectinate bristle projecting antero-laterad; laterad of this are 2 small, simple bristles and exteriad to these, 4 others, the most ventral of which is quadrifurcate; on the posterior lateral or metathoracic area are 4 curved pectinate bristles, all twice the length of those on the anterior or prothoracic area of the thorax. These bristles are all movable by means of voluntary muscles. The most ventral bristle has a small pseudopod at its origin. (Pl. III, fig. 3.) The same is true of the most ventrad of the anterior or prothoracic bristles and of a similar one medio-latero-ventrad on the mesothorax.

When a larva is examined ventrally it will be seen that the pseudopoda occupy a position relative to the six feet of a coleopterous or lepidopterous larva, a pair on the pro-, meso- and metathorax respectively. They are located on prominent, tumescent tubercles and are all capable of a slight motion in conjunction with the pectinate bristles. The middle and posterior pair are the only ones which show signs of articulation, each pseudopod being composed of two segments, not only visible in the living larva but also in the exuviæ and in macerated speciments. I am thoroughly convinced that these organs represent atrophied or degenerate organs of locomotion, not only because of their morphology, but also from their function, as observed in the living larva when it crawls over vegetation, or upon a rough, moist surface. I do not recall that any previous author has mentioned these organs and I call attention to them at this time in order to elicit a further discussion as to their origin and possible significance. I have noted the same organs in the larvæ of *Worcesteria grata* Banks and in those of certain other Culicidæ. I hope to present drawings of them in a paper dealing with the life histories of the Philippine mosquitoes.

Abdomen (Pl. I, fig. 3).—The *first* segment has a pair of lateral, approximate, pectinate setæ, curving forward as on the thorax; ventrad to this is a tuft of 3 simple setæ, submediad on the posterior area of the ventrum is a tuft of 3 short, simple setæ; the *second* segment is as the first, except that on the sublateral, posterior area is a tuft of 3 to 5 palmate hairs, laterad of which are 3 short, erect setæ in a transverse line; the *third* segment has a single, lateral, pectinate seta, a palmate-hair tuft consisting of 11 to 12 parts, 3 short setæ on a line anteriad of the palmate tuft and a small tuft of simple setæ posteriad of the pectinate seta, in addition to the ventral tuft; the *fourth* segment has a small, pectinate seta laterad and a subsidiary tuft of 3 to 4, simple setæ slightly posteriad. The palmate tuft on this segment contains 14 hairs and antero-laterad of this is a simple, erect seta, four times the length of the hairs in the tuft; the *fifth* segment is similar to the *fourth*, but its palmate tuft contains only 8 hairs; the *sixth* segment has several pale, scattering, simple setæ on the latero-dorsal area posteriad. Its palmate tuft is composed of 10 to 12 hairs. The *seventh* segment is similar to the *sixth;* the *eighth*, bearing the respiratory siphonette (Pl. I, fig. 6), has a few scattering setæ laterad. The *ninth* has a quadri-pectinate bristle, laterad, near its apex, and a series of 9 very long (twice the length of the segment) pectinate hairs, curving at their tips and articulated on a special, movable tubercle (Pl. I, fig. 6), the whole serving the double purpose of rudder and propeller in the locomotion of the insect. At the extreme caudal end of this segment, dorsad to the anal aperture are 4 palmate, brush-like setæ, the ventral pair of which has coarser branches than the dorsal, their number being 18 (in 9 pairs) and their extremities being curved. They are used by the insect for anchoring itself to plants on the surface of the water. The whole dorsum of this segment is covered by a single, chitinous sclerite (Pl. II, fig. 5) from the middle of each side of which projects a pair of simple, erect setæ.

The tracheal gills (anal gills) (Pl. I, fig. 6) are very short as compared with those of *Culex* and *Stegomyia* larvæ, which remain a long time beneath the water. These gills lie immediately around the anus and are about twice as long as broad.

The length of the larval period is from nine to thirteen days from the hatching of the egg to the transformation to the pupa. During this time the larva molts five times, the first stage being one and one-half to two days, the second two to three, the third two to three, the fourth two to five and the fifth two days.

The respiratory siphonette (Pl. I, figs. 3, 4 and 6).—This organ, situated on the posterior, dorsal area of the *eighth* segment, which is obliquely truncated to receive it, is of very peculiar structure and merits a detailed description. When open (Pl. I, fig. 4) it has the form of an irregular, elongated five-pointed star, the lobes of which are rounded. The anterior lobe is the largest and lies mediad. It is thin, mica-like in appearance, hinged at its base, and has a vitelline, globular tubercle at the median point of the base. This vitelline tubercle fits into a socket when the lobe is closed over the two tracheal apertures, each of which lies a little to one side of the median line. When the organ is closed, the two remaining anterior lobes fit against the respective posterior lobes, the latter doubling outward along their axes and inward along their points of union with the anterior lobes. The four lateral lobes close before the median, which fits against the others, effectually locking itself by means of the tubercle and socket and precluding the entrance of water. The entire structure is reënforced with strips of chitin running through various parts as shown by the darker lines in the figure. On each side of the siphonette is a lateral comb (Pl. I, fig. 6, and Pl. II, fig. 6) composed of fifteen teeth, those ventrad being longest and all pointed directly caudad. The comb is surrounded ventrad, anteriad and dorsad by a black, chitinous brace, from the dorsal cusp of which projects a quadrifid seta (Pl. I, fig. 7) four times the length of the comb teeth.

Pupa (Pl. IV, fig. 1).—The color of the pupa is dull gray, with a slightly greenish tinge. The last abdominal segment is light ocher. The first abdominal segment is darker dorsally than any succeeding one. The respiratory siphon is dark gray externally, the inner surface being pearly-white and striate. The eye-spots of the adult mosquito are plainly visible as dark areas on the antero-lateral region of the pupal cephalo-thorax.

The chætotaxy of the pupa is as follows, the hairs and bristles of but one side being given as in the larva: Submediad on the anterior margin of the metathorax is 1 small, simple erect hair, on the scutello-abdominal suture of the *first* abdominal segment, submediad, is a short, erect, bifurcate bristle, followed laterad by 2 simple bristles, the second of which is 3 times as far from it as the first; submediad on the middle of the *second* abdominal segment is a palmate tuft of hairs composed of about 30 simple and bifurcate branches; submediad on the *third* segment at its middle is a quadrifurcate bristle, followed laterad by a simple, stout, curved bristle; on the *fourth* segment is a long, trifurcate bristle, submediad on the posterior margins of the tergite, followed laterad by two other simple bristles, the first of which is anterior to the margin, the second on the margin; on the *fifth* segment there is a bifurcate, submedian bristle, followed laterad by a trifurcate bristle and antero-laterad by a bifurcate, smaller one; the *sixth* segment has a simple, submedian bristle, longer than the segment, on the posterior margin of the tergite, followed laterad by a quadrifurcate bristle, the *seventh* and *eighth* have bristles arranged similarly to those on the *sixth* except that on the *seventh* there is a very small hair, mediad of the long bristle, and its lateral bristle is bifurcate; on the *eighth* segment there is a trifurcate bristle anteriad of the long, simple bristle; the *ninth* segment bears a stout, dark brown, slightly curved bristle at the posterior lateral angle and a single, submedian, dorsal hair at the posterior margin.

Ventrally the *fifth* and *sixth* segments have a stout, latero-ventral bristle at their posterior margins, while on the *seventh* and *eighth* there is a submedian bristle on the posterior margin.

The caudal fins, or pinnura (Pl. IV, figs. 4, 5), have the central and external marginal veins brown at their bases, while the prolongation of the central veins, the urachætæ, are long, slender and recurved at their apices, forming a hook (Pl. IV, fig. 5). The external margins of the pinnura are fringed with delicate hairs from the urachætæ to the apex of the external veins.

Length of pupal-period.—The pupal stage lasts from two and one-half to three days according to weather conditions.

Habits of pupæ.—The pupæ, as a rule, remain at the surface in full sunshine, but swim very rapidly to the bottom or among or beneath the layers of algæ when any object comes near. They can remain submerged for from fifteen to forty-five seconds, but usually return to the surface almost immediately. Their pale gray color makes them very difficult to detect, and were they to remain quiet at the surface they would be well protected by the background of green or light gray of the decaying, matted algæ. No special or notworthy difference appears to exist between their position and that of the pupæ of *Culex microannulatus* Theob., with which they are often intimately associated. A mass of congregated pupæ will frequently contain individuals of both species in about equal number and the pupæ of *Myzomyia* are only distinguishable by their lighter color and slightly smaller size.

Myzomyia ludlowii Theob.

Description of adult female[4] (Pl. V, fig. 1).—Palpi deep brown, the apex broadly white-banded, another small band close to it and a third much lower down (Pl. VII, fig. 2); proboscis deep brown, with distinct, creamy-white tip. Thorax fawn-colored in the middle, dark brown at the sides, with a median and lateral dark lines and curved, hair-like, pale scales; abdomen brown, with pale hairs. Legs mottled and spotted with yellow; tarsi apically and basally pale, banded. Wings with 4 large costal spots and one or two small basal ones, most of the veins of wing area pale scaled. (Pl. VII, fig. 3.)

♀ Head pale brown, with narrow, pale scales and pale and brown, upright, forked ones, a pale, median tuft projecting forwards; antennæ brown, basal joint pale ferruginous; proboscis deep brown, apex creamy, palpi deep brown, with a broad, creamy, apical band, and near it another narrow, pale band, the remainder divided by another narrow, pale band, base densely dark scaled.

Thorax fawn-colored in the middle, dark brown at the sides, with a median, dark line and a narrow, dark line on each side of the pale area, with scanty, hair-like, curved, pale scales and traces of a dark spot before the scutellum, which is pale brown, with narrow hair-like scales; pleuræ brown, mottled with gray.

Abdomen brown, with narrow, curved, hair-like scales and pale posterior border-bristles.

Legs brown, the femora and tibiæ and metatarsi, especially in the hind legs, spotted with yellow; tarsi with broad, apical and basal, pale banding, especially on the hind legs; ungues small, equal and simple.

Wings with 4 large, dark, costal spots and two small, basal ones, the apical spot small, extending evenly on to the first long vein; this is followed by a pale area nearly twice as long as the black apical spot. The second black spot is about the same length as the preceding pale one, and spreads evenly on to the first long vein; the next pale area is slightly longer. The third black spot is the largest and spreads nearly evenly on to the subcostal vein, while beneath it on the first longitudinal is a large, black line and then a small, pale area followed by a small, black spot, the black line not beginning directly under the costal spot. The fourth black spot is separated from the third by a very small, pale area, and extends evenly on to the subcostal and first longitudinal; at the base is another small, black spot. The second long vein has dark scales on each side of the cross-vein and a dark spot on the upper branch of the fork-cell under the apical costal spot, and another small one near its base; the lower branch has an apical spot and a larger one near its base. The third long vein has a black, apical spot and a dark patch on each side of the cross-veins; the fourth is mainly dark on each side of the cross-veins, and has 2 dark spots on the upper branch, one near the base and two on the lower branch; the fifth has a black spot at its root, 3 on the upper branch and one at the apex of the lower branch; the sixth has 2 dark spots. The first submarginal cell is a little longer and decidedly narrower than the second posterior cell, its base slightly nearer the apex of the wing than that of the second posterior cell, its stem as long as the cell; stem of the second posterior considerably longer than the cell; supernumerary and mid cross-veins in one line, the posterior rather more than twice its length distant from the mid, but very variable, sometimes step-like; fringed with pale spots.

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[4] This description of the female adult (Pl. V, fig. 1) is taken from Theobald, *Mono. Culic.* (1903), 3, 42, while that of the male is prepared by myself, this sex having never been described.

Length.—4 to 4.8 millimeters.

Habitat.—Luzon, Philippine Islands (Miss C. S. Ludlow).

Time of capture.—April.

Observations.—Described from a number of specimens. A very variable species, somewhat like *Rosii* at first sight, but easily told by the spotted legs and much shorter fork-cells. The base of the first submarginal is always slightly nearer the apex of the wing, and the costal spots differ slightly, but are to some extent variable. The cross-veins are most unstable. The palpi are very similar, but the apical band in *Rossii* is rather longer. The chief difference is that in *Rossii* the second white band is a third of the way down the palpi; in this species it is less, and the black, intervening area is much smaller.

Description of adult male (Pl. V, fig. 2).—Pale-gray, the disc of the thorax being lighter than other parts of the body, the abdomen and pleuræ of thorax darkest. Head with dark-brown, erect scales at sides and white ones in middle of occiput. Eyes ruby-red, with curved bristles projecting over them anteriad from their posterior margins; frontal tuft long, cristate, prominent, antennæ pale golden-brown two distal segments dark-brown; the 3-jointed palpi long, slender, except the two apical segments which are tumescent (Pl. VII, fig. 1), with rounded apices and the penultimate having an internal tuft of long, golden hairs at its base; basal ⅖ of first papal joint dark-brown, followed by narrow, white band; apical ⅗ or less, dark-brown with a long, narrow, white spot covering its middle third and a transverse, triangular, white spot at its apex. The second segment all white above, except a narrow, brown area at its base, the entire under surface being brown also; the apical segment nearly all white, except a very narrow area of brown at its base, the proboscis dark-brown, except the tip which is lighter. The palpi and proboscis are of the same length. Around the lower rim of the eyes is a fringe of dark-brown hairs, pointing posteriad.

The denuded thorax shows one median and one submedian, thin, dark line on anterior ¾, the remaining surface being covered with a whitish pruinescence.

The costæ of the thorax are longitudinally striped dark brown and gray, there being approximately 13 or 14 stripes. The prothoracic lobes are well defined and bear a tuft of dark-brown setæ. The scutellum is perfectly nude, gray and with a transverse, median, dark brown spot; the meanotum nude, dark gray. Halters pale gray with brown knobs.

The abdominal segments uniformly dark mottled-brown with the entire surface uniformly decorated with fine, golden setæ. On the eighth segment and the harpes dorsally the surface is covered with white scales, in addition to the abundant, golden setæ.

The wings are as in the female, except that they are paler.

The legs are uniformly golden and brown mottled, the apices of the tibiæ, metatarsi and tarsi being pale, banded, especially the posterior tarsi which are themselves darker than the remainder of the legs.

The first anterior tarsal joint has a double row of short, stout, dark spines on its entire lower surface, there being about 108 in alternate succession. The empodium is ¼ the length of the fifth tarsal joint, its median seta making its total length ½ that of the joint. There is a small, blunt spur at its base.

The anterior tarsal ungues (Pl. VIII, fig. 1) are unequal, the larger being curved and having a single tooth at the middle.

Length of ♂ 5 millimeters, length of wing 3 millimeters.

Manila, P. I.

Time of breeding, 14 August, 1906.

Type ♂, No. 5744 in Entomological Collection, Bureau of Science, Manila, P. I.

Only the female of this species has been described previously by Theobald, and I find no mention of the male having been taken. All my males were obtained by breeding. They are about equally as abundant as the females in bred material.

I have noted that when at rest, the males fold their wings so that the costal edges instead of being parallel, as in the female, converge posteriorly, giving the insect a distinctly tapering appearance. This feature will be a sure guide to distinguishing the sexes in living specimens when they are at rest, and even when the palpi are not readily visible or distinguishable. The males feed readily on banana and suck large quantities of salt water, their abdomina often appearing almost transparent from the amount taken. The structure of the mouth-parts of the male is such that it is impossible for it to puncture the skin and therefore it can not bite.

HABITS OF THE ADULT.

Contrary to the general rule and to my expectations, this mosquito is essentially a dry-season form; that is to say, it is most prevalent during the months from November to June. I believe it to be a strictly salt-water form, delighting, during the larval and pupal stages, in the brightest sunshine. This explains its comparative scarcity in the rainy season. Its breeding grounds, because of the tides, never dry completely. The temperature during the dry period is very favorable to the multiplication of several generations. The lack of hard rains, which would beat the surface of the water in the breeding places, makes it possible for the greatest number of eggs to hatch and of larvæ to reach maturity, while the adults find sufficient shelter from the sun, which is fatal to them, in the dense foliage and along the banks of their breeding places.

If the adults are disturbed during the bright, sunny hours of the day, they invariably fly but a short distance, alighting in the characteristic attitude, always in the shade and usually upon the underside of a leaf.

These mosquitoes are frequently found in houses and their favorite resting places are the undersides of tables, cupboards, beds, in the dark corners of ceilings or upon the portions of dark clothing which are shaded. The females are the ones invariably encountered. It appears that the males never leave the vicinity of their breeding places.

I have observed that both sexes drink salt water when they are kept in confinement, usually within an hour after their emerging from the pupa.

Copulation.—The sexes are equally active and in the late afternoon they may be found copulating, both in the free state and in captivity. The mode of copulation is the same as that of *Stegomyia*. The females which hang from the leaf or the gauze over the breeding jars, loose their hold with their middle legs and hang only by the fore legs when approached by the males. The latter fly against them from the ventral side, entangling their legs with those of the female, the anterior pair being clasped around the thorax just back of the neck and the middle pair being sometimes apposed to those of the female, sometimes entangled. The act of copulation then takes place and may last as long as 30 seconds, the male flying away to another part of the jar and the female proceeding to preen herself after the manner of house flies. One male has been observed to copulate with four or five females within as many minutes. None of these mosquitoes have been observed doing so while on the wing, although it is not unlikely that this does occur.

Egg-laying.—The female when about to lay her eggs wanders on the wing just above a place where the algæ are partly out of the water. She settles down after a short time, walking somewhat rapidly over the surface, trying different places with the tip of the abdomen. During this latter process the body is about parallel with the surface of the water, while the head and palpi are inclined downward. This is apparently the only time in this mosquito's life as an adult when her head and proboscis are not in a line with the main axis of the body. When the ovipositor encounters a small particle either of the algæ or of other material projecting a short distance above the surface film, the female stops, palpates with the tip of the body and almost immediately oviposits, going thus from point to point till she has laid from ten to twenty-five eggs, she then stops for a time to preen herself and to rest. Sometimes as many as seven or eight eggs will be laid in a place. As the females which laid their eggs in captivity did so only after having been fed a meal of blood, I suppose that those observed in nature had also procured such food, although at the time of the observations their bodies showed no signs of having blood within them. This may be due to the fact that the eggs are laid some five or six days after the insect has fed. Those mosquitoes which were observed laying in captivity were seen to have white masses of eggs within the abdomen posteriorly, just before they were observed to begin ovipositing.

Habits of the female.—The female is much more active than the male after copulation, which occurs after thirty-six hours of emergence. Although she remains quiescent during the day, she is very alert after 5 o'clock in the afternoon. In both sexes the position of the body is even more pronounced than that shown in drawings and sketches of Anophelinæ by various authors. In some instances individuals are seen which have the body nearly at a right angle to the surface upon which they are at rest and the posterior pair of legs is bent up over the back so that the tarsi are not very remote from the dorsum of the thorax. The female when disturbed flies off very rapidly in a straight line, and if annoyed when in the act of inserting her proboscis for biting, she almost invariably returns to the same point to renew the attack. When resting upon a wall or other object the females of *Myzomyia ludlowii* Theob., are not difficult to capture in very small vials, provided the extended hind legs are not touched. These limbs are evidently intended to serve the same purpose as the rostral hairs of animals like tigers, cats or mice. They are kept in almost constant motion, sometimes one is elevated, more frequently both. The males are somewhat more wary than the females.

Blood-sucking.—The females of these mosquitoes attack readily in the evening; during the day it is not easy to persuade them, even when the receptacle containing them is covered with a black cloth. When one of the insects settles upon a chosen spot, its palpi are immediately elevated and the stylets of the proboscis inserted, sometimes to within less than a millimeter of their base. During the process of inserting the stylets, the female wriggles her palpi in a manner which seems plainly to suggest the pleasure which the act affords. After she has begun to draw blood, the motion of the palpi decreases somewhat, but does not entirely cease until she has completed the meal and withdrawn her proboscis.

An invariable habit of these mosquitoes while feeding on blood for the first time is that of voiding a serum-like liquid from the anus in a continuous stream of droplets. This is succeeded by pure blood with the corpuscles intact. An amount equal to that retained in the body will frequently be expelled in this manner in five or six minutes from the time the insect begins to bite. These dejecta retain all the microscopic appearances of uningested blood, excepting that the corpuscles are fewer in number, the greater part having been retained, probably in the stomach. All dejecta, subsequent to this peculiar one, are of the normal flyspeck kind, and contain the usual waste products of digestion. It appears as if the first meal of blood which the insect takes, acts as a very rapid cathartic and prepares the system for the digesting and assimilating of succeeding meals.[5] Unfortunately, no observations were made upon mosquitoes fed, previous to sucking blood, on the juice of bananas.

Susceptibilities and idiosyncracies.—Female mosquitoes of this species stand confinement very much better than males, the latter dying in from two to four days after emerging, even when abundantly supplied with food which they appear to enjoy. The females may be kept alive for at least twenty-three days by feeding them on blood every alternate day. It has been stated that unmodified sunlight is fatal to the adult mosquito, but the males succumb to such conditions very much more rapidly than the females. They are in every way more fragile than the other sex. They have never been seen to gorge themselves, either with juice from ripe bananas, water or sugar sirup, as do the females, and of course they *positively* do not suck blood. This has been thoroughly demonstrated by trials with more than five hundred male mosquitoes under every condition which could arise. If they can be directed to a drop of blood placed on the side of the vessel containing them, they will sip it up daintily for a short time, but never remain at it, as do the females, until the last bit has been drawn up.

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[5] It may be that the saline character of the blood gives it this cathartic effect.

MOSQUITOES AND MALARIAL PARASITES.

The examination of some five hundred blood smears from about fifty patients in the "naval sick quarters" at Olongapo demonstrated the presence of both tertian and æstivo-autumnal parasites, the former comprising about 65 per cent of the cases, the latter 35 per cent. In no instance was the parasite of the quartan infection positively identified, either microscopically or clinically. Abundant material was therefore continuously at hand for purposes of experimentation, and there was not a single case in which any of the men objected to allowing themselves to be bitten as many times as I desired. Many of the men were examined only once, but a number had fifty to sixty slides made from their blood in various stages of the development of the parasite and several of them were bitten by not fewer than twenty-five to thirty mosquitoes, this, of course, including some who were bitten as often as twelve to fourteen times by the same insect.

METHODS OF BREEDING.

Two methods were used for obtaining the adult mosquitoes for purposes of biting, one being to construct a cage over the breeding pond and to take the adult females during the early morning; the other to collect many of the mature pupæ at about 4 o'clock in the afternoon and to wait for the emergence of the insects on the same evening, which in the great majority of cases took place between 6 and 8 o'clock, although in fewer instances the time was retarded until the next morning. It was found that those emerging at eventide were ready and willing to bite by 9 or 9.30 o'clock at night, but that those which appeared in the early morning seldom were ready to attack until the succeeding night after 6 o'clock.

As soon as the adult mosquitoes emerged and had dried sufficiently to be able to take flight, they were removed from the breeding receptacle directly into glass tubes 16 centimeters long and 4 centimeters in diameter, over one end of which were fastened, by means of rubber bands, pieces of plain gauze. Wads of absorbent cotton were pressed into the other ends of the tubes, but not too tightly to prevent a circulation of air. When in the tube, the mosquitoes generally rested upon the gauze and this was sprinkled daily with water, which the insects drank each time it was applied. I attempted to observe whether the insects would copulate in the tubes, but have never seen them do so, although eggs developed in one or two females, so that fecundation had probably taken place. In placing males and females into the tubes, care was taken to select only those females which had not copulated in the breeding jar. They were carefully watched during emergence and removed, in some cases, even before the integument had dried. None of these mosquitoes were used for breeding purposes.

INOCULATION OF MOSQUITOES.

At the beginning of the work numbers of mosquitoes were fed upon blood from persons negative for the malarial parasite, and I also allowed several of the insects to bite me. The mosquitoes were dissected at intervals of from eighteen hours to several days after biting, for the purpose of becoming perfectly familiar with the appearance of the internal organs, including the stomach, the thoracic cavity, muscles, and the salivary glands under normal conditions. After this had been done and the technique thoroughly outlined, patients were selected whose blood had, upon examination, shown the greatest number of parasites of the æstivo-autumnal type, which was chosen because of its great prevalence among the troops and the presence in the hospital of men who at the time were well infected by the crescent forms. The tubes used to carry on the work were prepared so that some contained a single insect, others held six and others twelve; those with but one being kept for voluntary cases who would submit to being bitten by the infected mosquitoes. The others contained insects which were to be allowed to bite malarial persons and then were to be placed in alcohol or dissected at various periods in the development of the parasites.

Dissection of mosquitoes.—Six hours after the mosquitoes had first bitten the patient, the insects were dissected and, from the blood within the ventral reservoir, smears were made upon slides, fixed and stained with Wright's stain after the usual method. At first some difficulty was experienced in obtaining a satisfactory adhesion of this blood to the slides. It was found that by either heating alone, or fixing with absolute alcohol and ether alone, the blood would largely be removed when it came to staining the slide. Finally, a combination of the two processes was used, heating first and treating with alcohol-ether afterwards being resorted to.

The crescents and ovoids were demonstrated in the blood obtained as above, but, unfortunately, mosquitoes in this stage were not placed in alcohol, so that no specimens of this part of the phenomenon were preserved except on the smears of blood taken from the stomachs of the insects.

Mosquitoes were dissected daily from the time of the first ingestion of blood, but nothing of interest was noted in the unstained tissues up to the day when the swellings in the gastric mucosa indicated the probable growth of the oöcysts. The fourth and fifth days' dissection demonstrated a considerable enlargement of the oöcysts, which attained a diameter of from 30 to 60 μ. On the sixth and seventh days they were for the greater part found as collapsed bodies on the cœlular surface of the stomach wall.

Sectioning of mosquitoes.—Further tracing of the parasites in any but sectioned specimens was impracticable. Mosquitoes were therefore put into alcohol and sent to Manila for sectioning, so that all stages of the passage of the parasites to the salivary glands might be traced. By this means their presence in all the intervening tissues between the stomach and the salivary glands was demonstrated. The accompanying photomicrographs (Pl. IX, figs. 2, 3, and 4, and Pl. X) show them also in the interstices between the muscle-fibers of the thorax, and in the salivary glands themselves.

Appearance of the sporozoites.—Specimens stained with hæmatoxylin-eosin, prepared according to the ordinary formula, show the normal muscular, glandular, and connective tissues stained red or blue, while within the cell structure itself innumerable, extremely small granules, either globular or elongate, appear, measuring not more than 0.75 μ in diameter and of a dark brown color. They are not only found within the cells of the tissue, but also, as is shown by certain salivary glands examined, adherent to the external walls of the gland cells. This phenomenon is shown in Plate IX, fig. 4, at the point marked *spor.*

TRANSMISSION OF THE PARASITE.

Upon going to Olongapo, it was not my intention as it was not my hope to procure volunteers who would be willing to submit to being bitten by mosquitoes infected with malarial organisms, so that I said nothing except in a suggestive way to the gentlemen in charge of the medical work at this station, until I had reached to the stage of demonstrating the oöcysts in the stomach wall of the mosquito. Finally I obtained one volunteer, the result being as follows:

CASE I. *Volunteer malarial infection.*—J. C. B. had been in the Philippines nearly two years; his medical history during his six years' service in the Navy gave no indication of his ever having had malaria. His statements were also to the effect that he had never had malaria, chills and fever, or any illness that would point to his having been infected. Many examinations of his blood demonstrated continuous absence of malarial parasites.

The patient was first bitten by a malaria-infected mosquito at 5 p. m. of June 1, 1907. This mosquito did not draw any blood, after having repeatedly thrust its proboscis into the patient's arms at different points. On the morning of June 2 at 8 he was again bitten by the same mosquito and by two others which were confined together in another tube. At 10.30 on the morning of June 3, at 10.30 a. m. of June 4, and at 8.30 a. m. of June 6 he was rebitten by the same three mosquitoes, they having had no other alimentation beyond that furnished by his blood. Every day, except on June 8, he showed a slight rise of temperature, but never above 37.4° C. On June 9 the patient complained of malaise, severe headache and chill, which latter was not very marked. Temperature at 12 o'clock, 38.3° C. In the evening the patient again felt well and during the next day his temperature was practically normal. On June 11 the temperature rose to 38° C., the patient having a slight chill, although he did not lie down nor desist from his routine work. From June 11 until the time of my departure, he had no further rise of temperature and no chill; he, however, continued his chart up to and including July 6. It shows that on June 24 he had a slight chill and that on the next day his temperature was 37.7° not rising again until June 28 when it reached 38° C., and again on July 2, 38.2° C. On July 1 the patient had headache, nausea, and general listlessness, with loss of appetite and frequent dizzy spells. On September 4 he reported as follows: "I had a spell of fever commencing with August 3 to August 14. I kept track of my morning and evening temperatures * * * noting them down in my * * * journal The afternoon of August 3 I had a chill and a temperature of 38.5°, then around 37.2° and 38.3° C. until August 6 when I had another chill and a temperature of 39.2° C., then fever off and on till the 14th of August."

Examination of blood from the patient.—On June 3 three slides were taken at 8 o'clock. Transparent, motile amœboid bodies moving freely from one red corpuscle to another were observed in a glass cell. They were evidently parasites which were too debile to enter the corpuscles. Nothing was noted on June 4. On the morning of June 5 the same bodies were seen in the blood serum. They appeared to move with greater vigor. The eosinophiles had attached large numbers of these bodies to themselves and in many instances they had engulfed them. On June 6 a stained slide gave a blue body within the pink corpuscle, but its identification was not certain. On the morning of June 7 the first positive identification of a parasite was made. It was half grown, of the æstivo-autumnal type. It was moving very slowly around an axis without appreciably changing its shape. The nucleus was plainly visible when stained and pigmentation had just begun. Another slide was taken on the same morning and stained by Wright's method. It demonstrated several parasites in the half-grown stage. On June 9 the parasites were again positively identified, also on June 10 to 12, but not on the 13th. On June 14 parasites were observed in the presegmenting stage, and on June 15 and 16 they were seen in all stages up to that of presegmentation. On June 17 a perfect æstivo-autummal pre-crescent ring was found, as also on the 18th, which was the last day of taking the blood.

A noteworthy feature of these blood examinations was the great numbers of parasites that were included by the phagocytes. Parasites which were seen to attack a red corpuscle at one moment would be engulfed the next by an eosinophile, although the phenomenon of the parasite entering the red corpuscle was frequently observed.

It should be borne in mind that the patient was bitten by infected mosquitoes up to June 6, so that naturally his blood would present an increasing number of parasites in the peripheral circulation toward the latter part of the experiment.

CONCLUSIONS.

The work on transmission performed upon the case cited above was in every way of such a positive character, the after effects so marked and the previous history of the patient so well ascertained that there remains no doubt in my mind as to the validity of the tests made. However, it seems very desirable, in order to subject this question to further concise test, that a number of volunteers be secured to undergo, in properly constructed cages, an isolation period followed by inoculation by *Myzomyia ludlowii* Theob., and as soon as the season of abundance for these mosquitoes returns I shall carry out further experiments looking to this end. The results of the continuous experiment at Olongapo have been published at this time as it may not be possible to furnish further proof until several months have elapsed, and it was not thought desirable to delay the publication of the work so far done.

ILLUSTRATIONS.

Map of Olongapo, Zambales, showing United States naval station and surrounding region with breeding places where *Myzomyia ludlowii* Theob., was actually found, indicated by red cross lines.

PLATE I.

FIG. 1. Egg of *Myzomyia ludlowii* Theob., top view, showing micropyles. 1×25.

2. Egg of *Myzomyia ludlowii* Theob., side view, showing large and small air-chambers. 1×25.

3. Full grown larva of *M. ludlowii* Theob. 1×10.

4. Respiratory aperture of siphonette of larva, showing vitelline tubercle in center of upper ray with the corresponding socket near middle of star and tracheal openings in upper part on each side. 1×40.

5. Right mandible of larva, showing dentation and auxiliary bristles and palpuli. 1×240.

6. Eighth and ninth abdominal segments of larva, showing (*A*) anchor bristles, (*B*) respiratory siphonette, (*C*) lateral comb, (*D*) tubercle for propelling setæ, (*E*) anal gills, and (*F*) propelling setæ.

7. Quadrifurcate seta from comb, the type of numerous setæ distributed over various parts of the body of the larva. 1×240.

PLATE II.

FIG. 1. Head of full grown larva, showing chætotaxy and markings. 1×60.

2. Labium of larva, with base of clypeus. 1×400.

3. Maxilla of larva, showing external, multiple seta and palpus. 1×400.

4. Ventral and lateral aspects of the labial plate of larva, the lateral aspect showing its double laminate structure. 1×400.

5. Dorsal chitinous sclerite of ninth abdominal segment of larva, showing paired anchor setæ (the outer ones) and propulsory setæ. 1×400.

6. Lateral comb from eighth abdominal segment of larva with chitinous brace and part of quadrifurcate seta. 1×400.

PLATE III.

FIG. 1. Head and thorax of larva in penultimate stage showing great width of colored, basal band on head. 1×60.

2. Dorsal aspect of thorax of full grown larva, showing chætotaxy. 1×60.

3. Ventral view of thorax, showing pseudopoda and chætotaxy. 1×60.

PLATE IV.

FIG. 1. Lateral aspect of pupa, showing chætotaxy and respiratory siphons. 1×12.
2. Lateral aspect of respiratory siphon. 1×25.
3. Dorsal aspect of respiratory siphon (1×25), showing opening lined with short, fine hairs which preclude entrance of water.
4. Dorsal aspect of abdomen of pupa, showing second to ninth segments and pinnuræ or caudal fins. 1×12.
5. Pinnura or caudal fin, showing form and position of urochæta. 1×25.
6. Perspective of abdomen of adult female mosquito, showing ovipositor and chitinous sclerites which compose the ninth segment. 1×60.
7. Perspective of abdomen of adult male mosquito, showing harpes and hairs together with sclerites which compose the ninth segment. 1×60.
8. Arrangement of supernumerary and cross veins in wing of male. (1×150): I. Supernumerary. II. Mid cross vein. III. Posterior cross vein.
9. Arrangement of supernumerary and cross veins in female. 1×150.

PLATE V.

FIG. 1. Adult female of *Myzomyia ludlowii* Theob. 1×8.
2. Adult male of *Myzomyia ludlowii* Theob. 1×8.

PLATE VI.

FIG. 1. Adult male of *Myzomyia ludlowii* Theob. Note position of claspers which is characteristic. This photomicrograph, as also the following, is made from a balsam preparation of a specimen treated with 5 per cent potassium hydroxide.
2. Adult female of same. Note the extruded spermatheca below the ovipositor. This organ lies normally within the eighth segment. The scutellum appears in profile as a knob at the base of the right wing.

PLATE VII.

FIG. 1. Eyes, antennæ, palpi, and proboscis of male *Myzomyia ludlowii* Theob. Note the construction at the middle of the second palpal segment, representing ankylosis of two segments.
2. The same of the female, showing the lancet and other punctorial setæ removed from the proboscis or labium.
3. Wing of *Myzomyia ludlowii* Theob., showing spots (female).
4. Wing showing arrangement of cross veins in the female.
5. Three ultimate abdominal segments of female showing spermatheca in position.
6. Eighth abdominal segment showing circi-like ovipositor with squamation.

PLATE VIII.

FIG. 1. Ungues of forefoot of male.
2. Ungues of forefoot of female.
3. Ungues of middle foot of male.
4. Ungues of middle foot of female.
5. Ungues of hind-foot of male.
6. Ungues of hind-foot of female.

PLATE IX.

FIG. 1. Genitalia of male of *Myzomyia ludlowii* Theob.

2. Section of thorax of infected mosquito, showing group of sporozoïtes of malaria at *A*. 1×1600.

3. Section of thorax of mosquito in vicinity of salivary glands, showing migratory sporozoïtes. 1×1600.

4. The lobes of salivary glands of mosquito with sporozoïtes thickly congregated around the upper one of them at *spor*. 1×1600.

PLATE X.

FIG. 1. Section of salivary duct of mosquito, showing agglomeration of malarial parasites with meganucleated cells in upper right. Best group of parasites at *A*. 1×1600.

2. Section of thorax of mosquito, showing sporozoïtes migrating through intermuscular spaces. 1×1600.

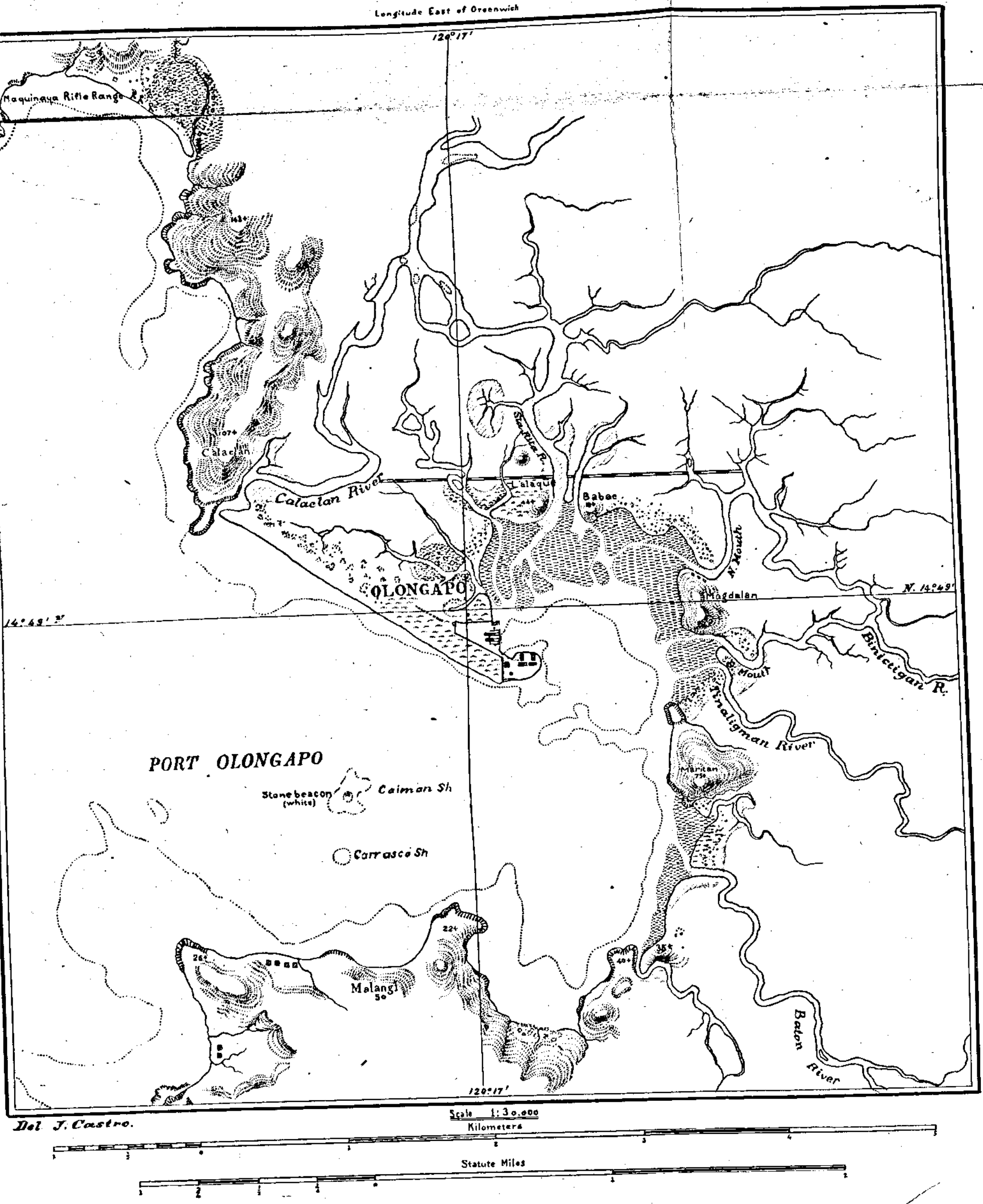
PLATE XI.

Clinical chart of J. C. B., upon whom experiments in inoculation with malarial parasites were performed.

MAP OF OLONGAPO, ZAMBALES, P. I.

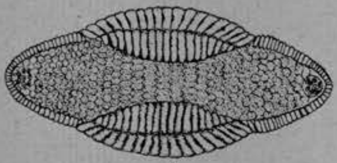
PREPARED IN CONNECTION WITH STUDY OF BREEDING
PLACES OF *MYZOMYIA LUDLOWII THEOB.*,
APRIL-JUNE 1907.
BY CHARLES S. BANKS, ENTOMOLOGIST,

BUREAU OF SCIENCE



Del. J. Castro.

Heights of hills in meters.



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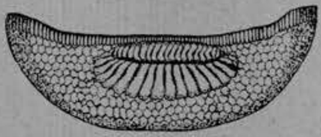

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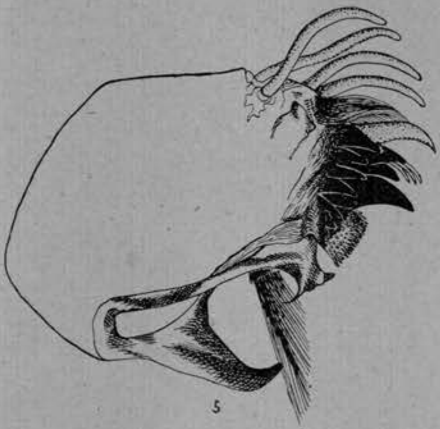

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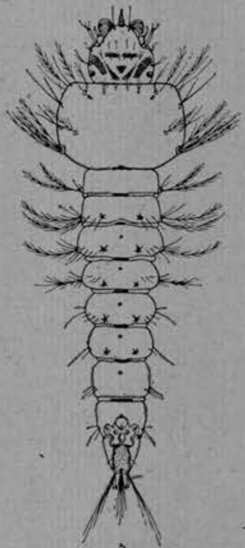

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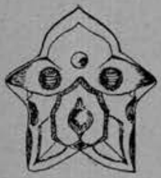

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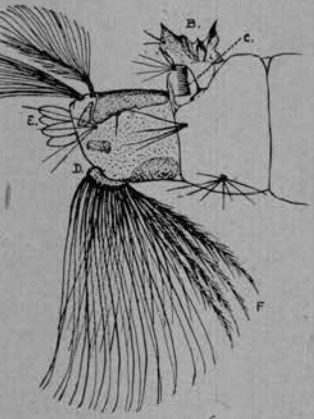

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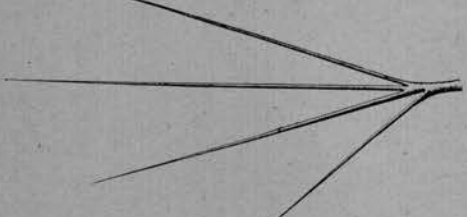

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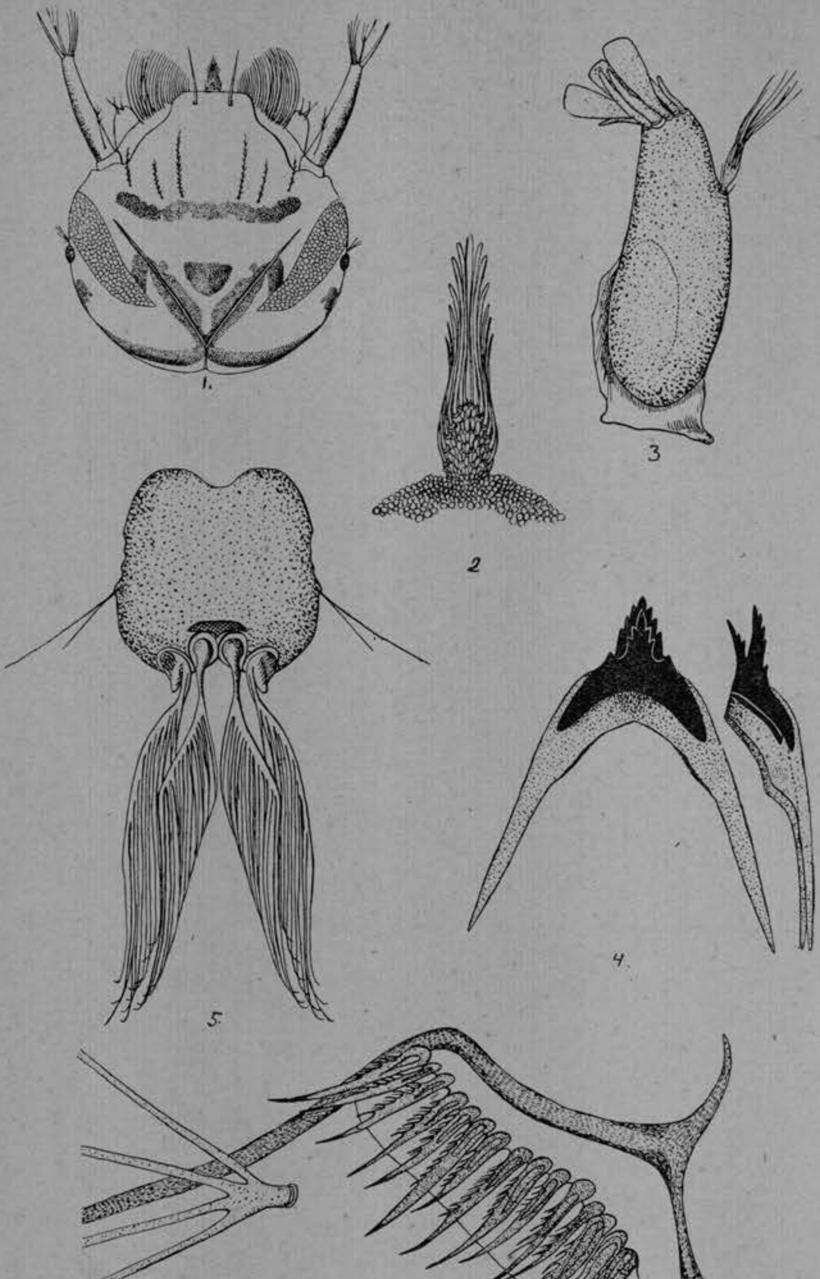

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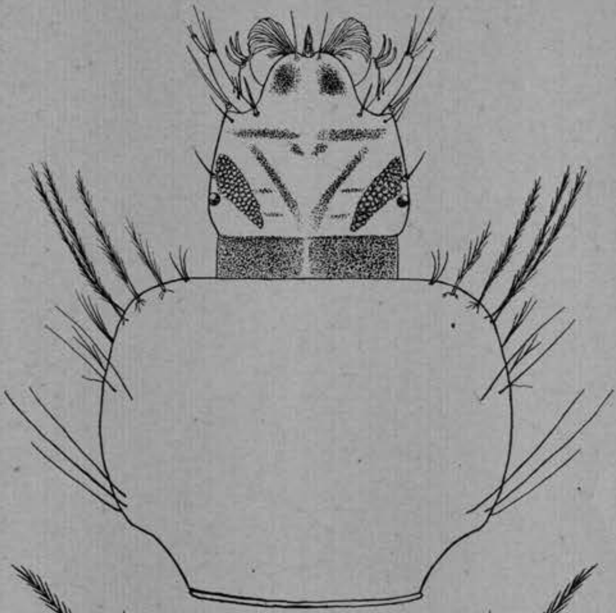

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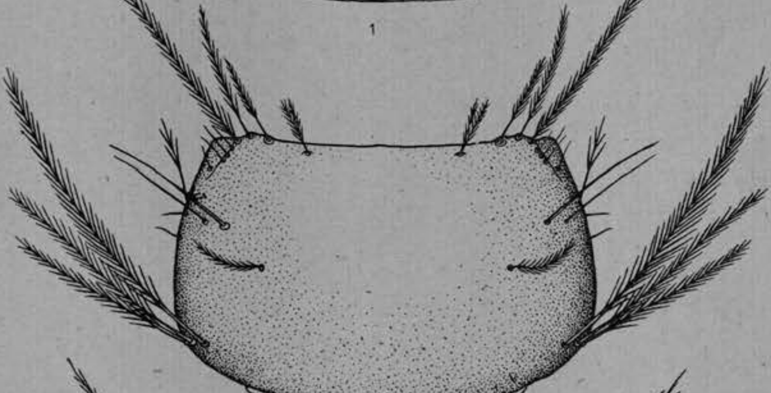

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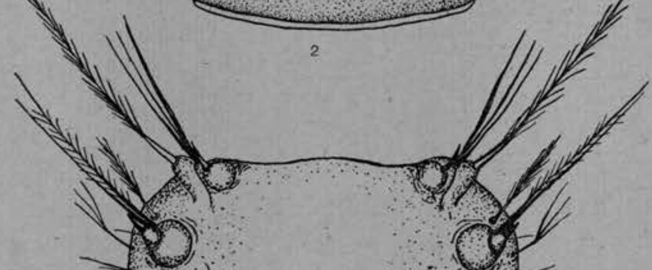

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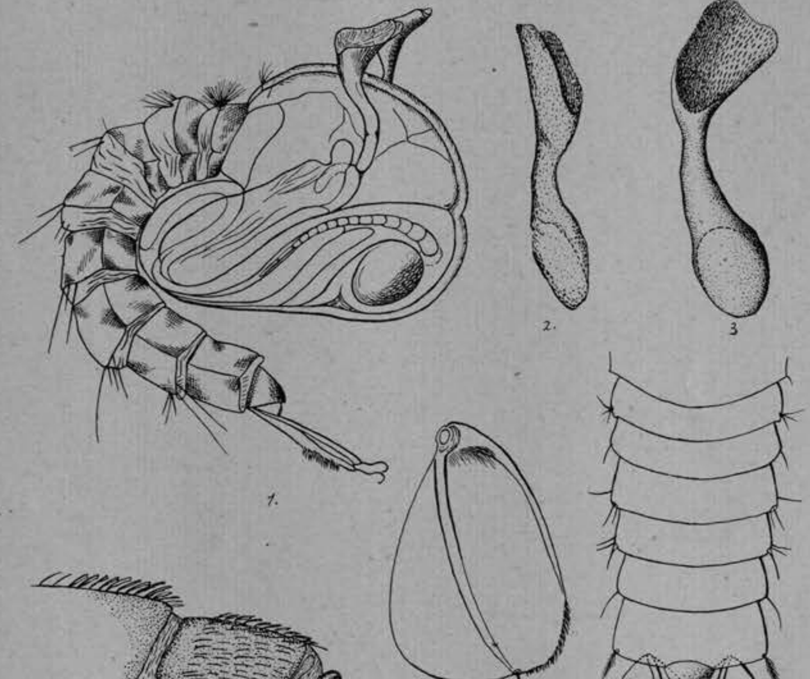
PLATE III.



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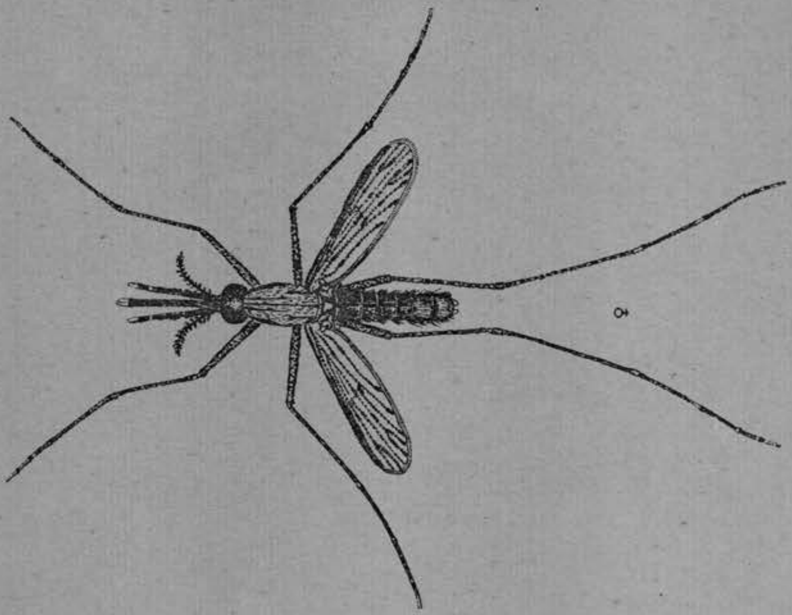

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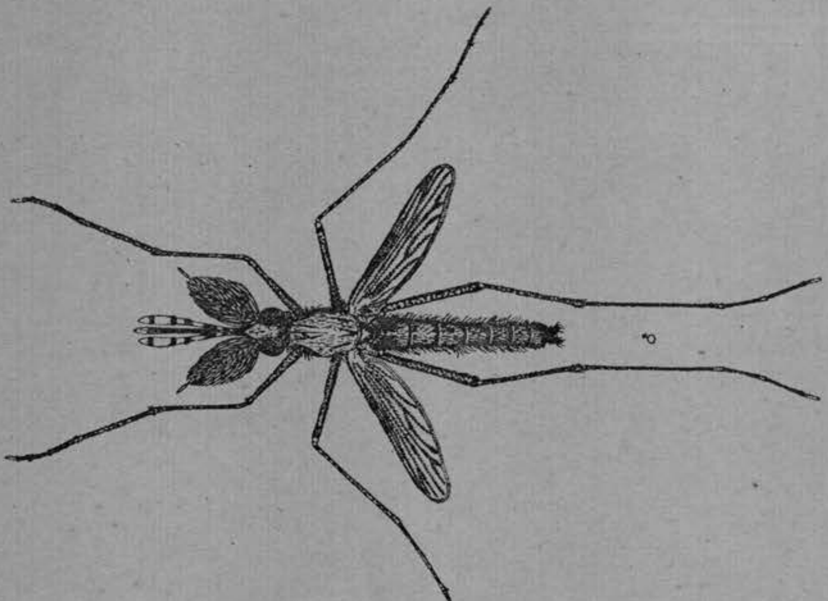
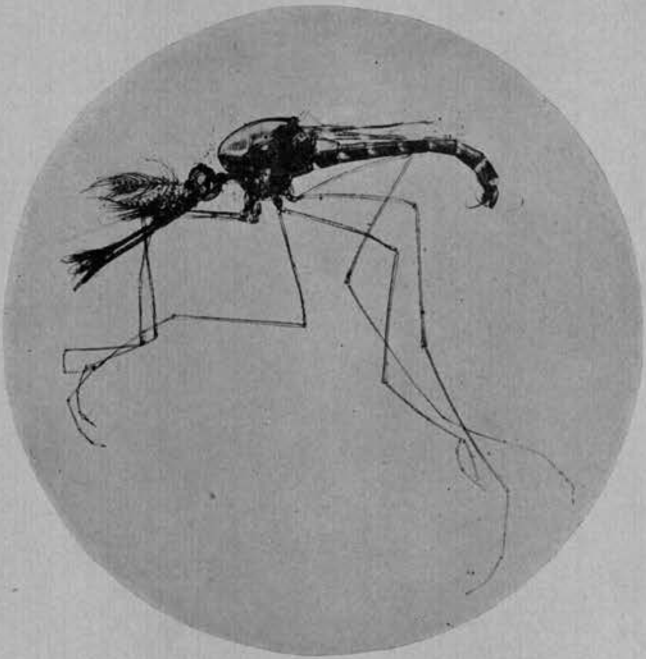


FIG. 2.

PLATE V.



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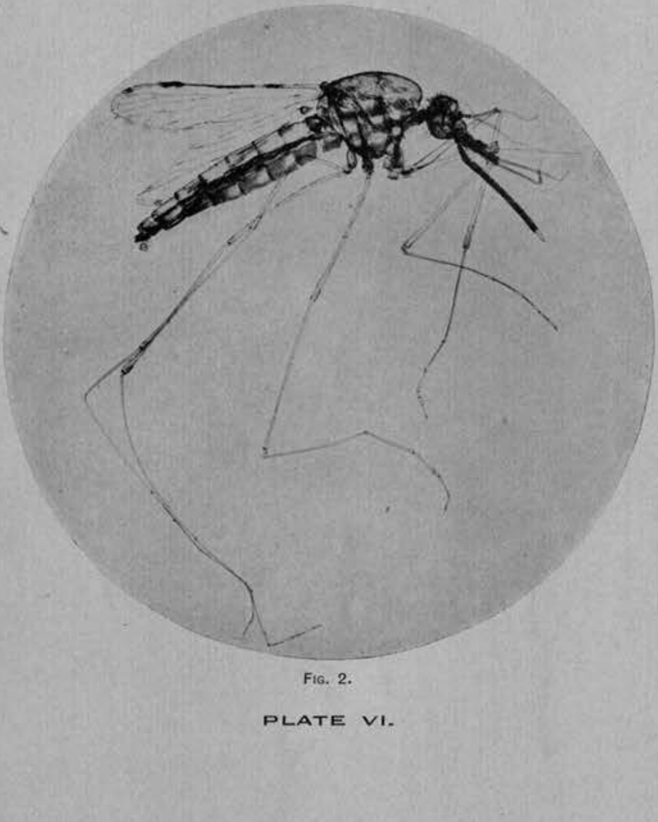
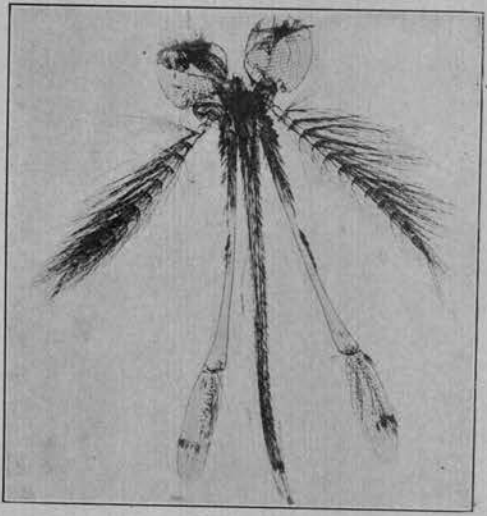


FIG. 2.

PLATE VI.



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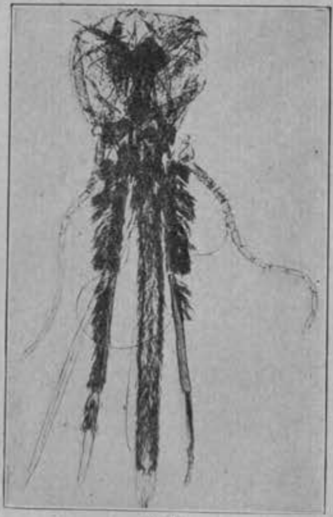

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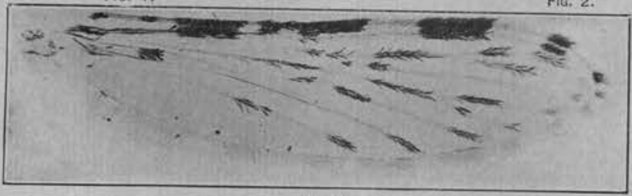

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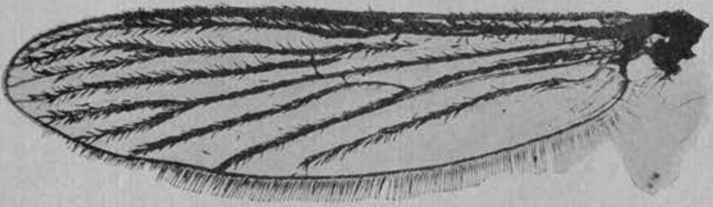

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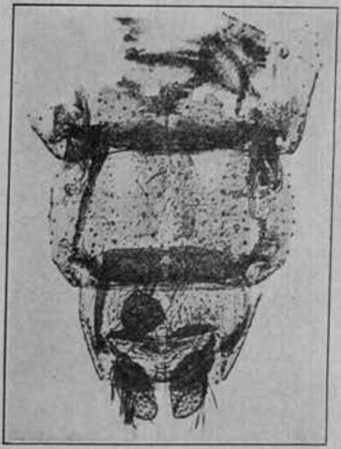

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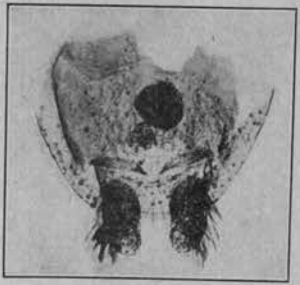
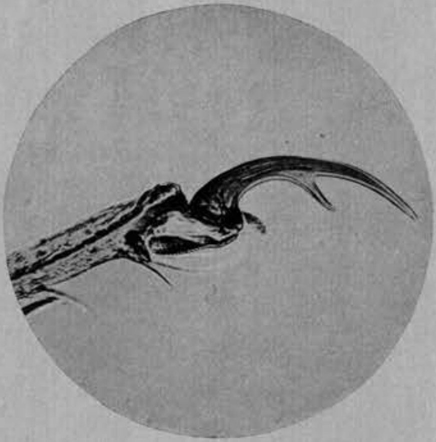


FIG. 6.

PLATE VII.



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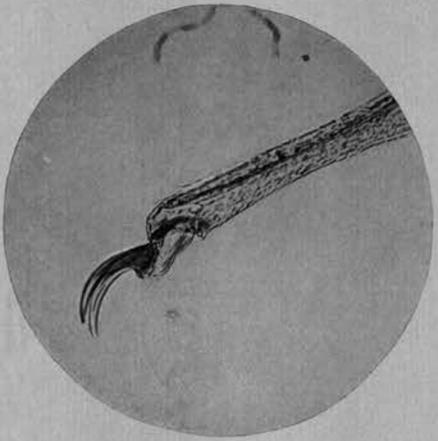

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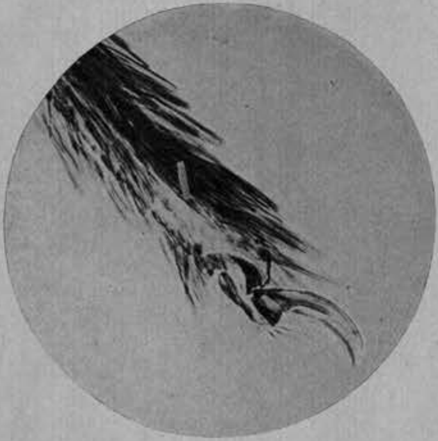

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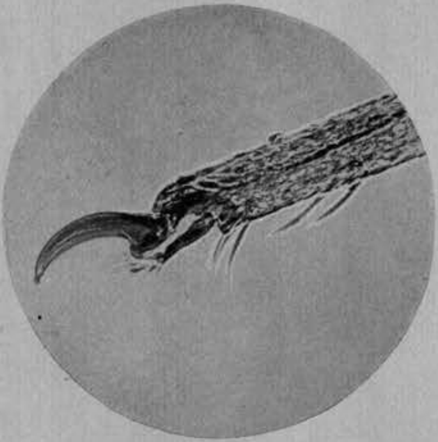

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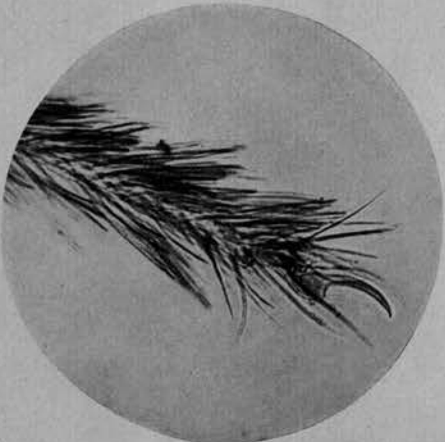

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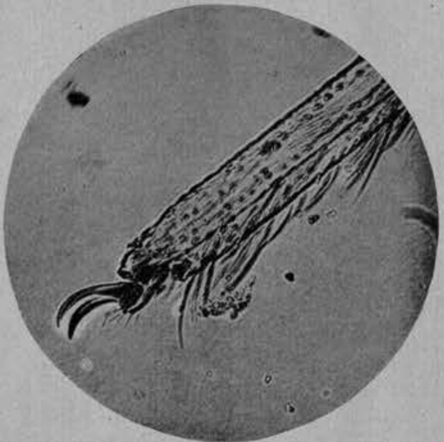
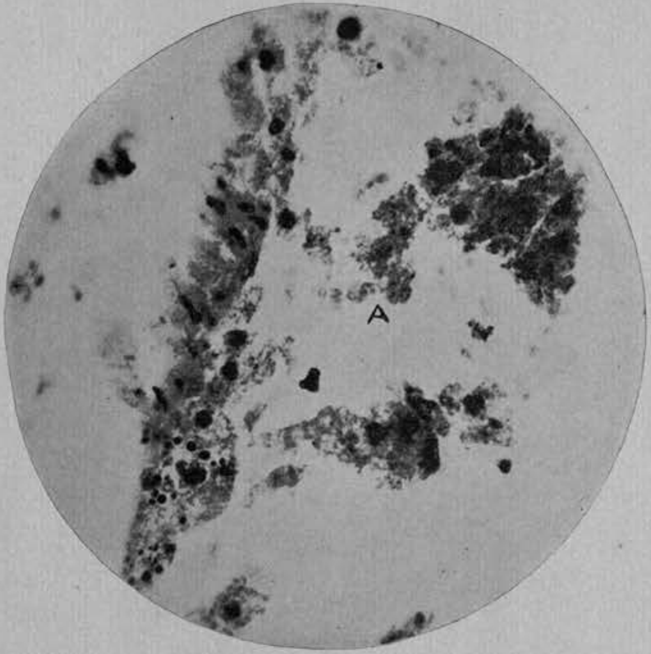


FIG. 6.

PLATE VIII.



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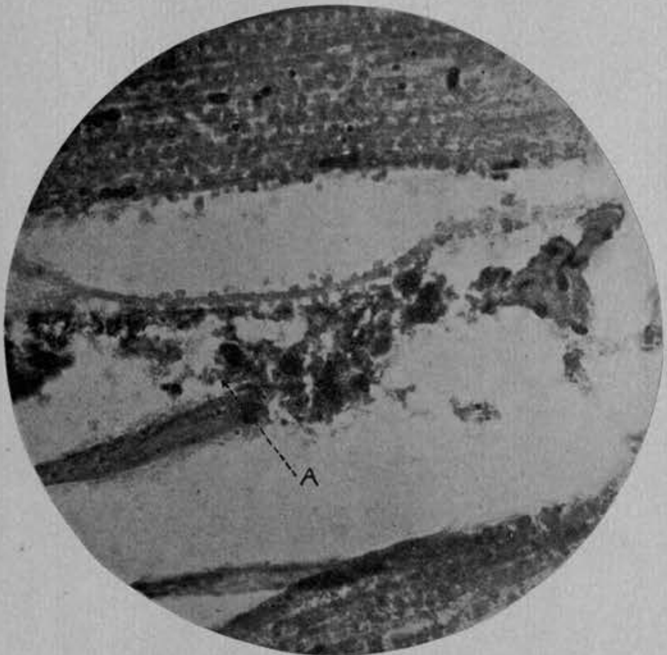
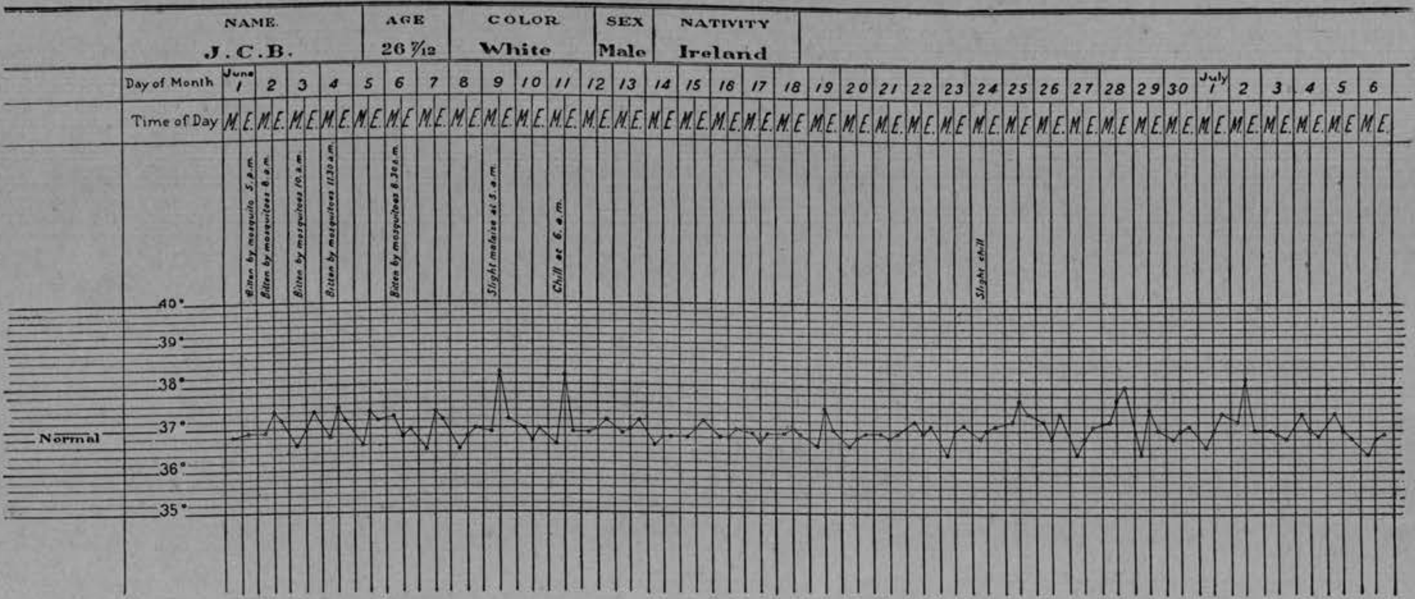


FIG. 2.

PLATE X.

PLATE XI.



Temperature Range 36 days-38.3°-36.4°-1.9°

A PRELIMINARY REPORT UPON THE SPECIFIC IDENTITY OF THE CESTODE PARASITES OF MAN IN THE PHILIPPINE ISLANDS WITH A DESCRIPTION OF A NEW SPECIES OF TÆNIA.

By Philip E. Garrison.[1]

(*From the Biological Laboratory, Bureau of Science, Manila, P. I.*)

INTRODUCTION.

The present paper embodies the results of careful determinative work upon such cestode material as has been collected for the Helminthological Collection of the Philippine Bureau of Science and is intended as the first of three or four papers, to appear as soon as the specimens in other groups can be properly worked over.

It is my purpose to endeavor to present in a systematic way some definite knowledge with regard to the specific identity of the more common animal parasites of man in the Philippine Islands. While the primary object of these publications is to clear the field for future work in the localities in question, rather than to present new helminthological findings, they naturally will include the description of forms coming to my attention in this preliminary work which seem to justify the establishment of new species or other systematic groups.

Our present knowledge of Philippine helminthology has been derived from a few determinations made by professional zoölogists upon material received from the Islands, from the reports of infections in Americans recently returned from a more or less prolonged residence in the Philippines, and from the work of resident medical scientists. While the information supplied by these sources has in many cases been lacking in the positive and precise identification of the parasites reported, it has established with reasonable certainty the presence in the Philippines of several forms already known in other parts of the world and has included the description of two new species of human entozoa, besides contributing considerable knowledge concerning the general prevalence of animal parasites in the Islands.

A summary of any results already published will be given in the consideration of each genus and species reported.

---

[1] Assistant Surgeon, United States Navy; detailed medical zoölogist to the Bureau of Science, Manila, P. I.

MATERIAL.

The greater part of the material forming the basis of the present study has been collected from native prisoners in Bilibid Prison, a large proportion of whom were examined and treated in quarantine immediately upon their arrival from various provinces scattered throughout the Islands. Numerous other specimens have been received from patients in the private practice of physicians and in the hospitals of Manila.

The present reports deal simply with the identification of specimens collected and they do not undertake to consider the proportion of persons infected to those examined; therefore they can not properly be taken as an index to the frequency of infection in the population. However, considering that from 3,000 to 4,000 persons gathered from widely scattered localities have been examined for animal parasites at Bilibid during the current year, and that specimens have been received from various classes and conditions of social life, it would seem fairly probable that our Helminthological Collection at present contains representatives of most of the species of the parasites of man which are generally prevalent in the Islands, and furthermore that, with certain exceptions which will be noted in the text of this and the following papers, the collection may be accepted tentatively as a rough indication of the relative prevalence of the different species.[2]

CESTODA.

The relative infrequency of cestode infections compared to infection with the common nematodes, and on the other hand the greater familiarity with tapeworms and their relative innocuousness compared with trematodes, are probably responsible for the fact that, while valuable contributions have been made to the study of the *Nematoda, Trematoda,* and the *Protozoa* of the Philippines, the *Cestoda* have been almost entirely neglected.

So far as we have been able to discover, the following are the only published references to cestode infections in the Philippines:

Strong,[3] 1901, reporting the results of 1,793 stool examinations and 386 necropsies, states that only two adult *Tænia* had been found, but that both *T. saginata* and *T. solium* are present in the Islands, the former species being most frequently seen in American troops, the latter occasionally occurring among the natives.

In the annual reports of the Superintendent of Government Laboratories of the Philippine Bureau of Science for the years 1902 to 1905, inclusive,[4] in which Dr. Strong, Director of the Biological Laboratory, includes the results of over 6,000 microscopic examinations of faeces, only five cestode infections are reported, all with the genus *Tænia*, species not noted. Hallock,[5] at Fort Porter, Buffalo, N. Y., reported two infections with *Hymenolepis nana* in American soldiers recently returned from the Philippines. Stiles and Garrison,[6] at the Government Hospital for the Insane, District of Columbia, found no cestode infections in making microscopic examinations of the stools of 115 American soldiers who had returned from Philippine service. In 1906, Foster[7] reported two infections with *Hymenolepis nana* in American soldiers on duty near Manila.

---

[2] I wish to acknowledge my indebtedness to Dr. Edwin C. Shattuck, resident physician of Bilibid Prison, for numerous specimens placed at my disposal and deposited in the Bureau of Science Helminthological Collection.

[3] *Circulars on Tropical Diseases,* Manila (1901), 1, 15.

[4] *An. Rep. of the Superintendent of Government Laboratories,* Report of the Biological Laboratory, Manila (1902), 569; (1903), 413; (1904), 443; (1905), 357.

During the past nine months there have come to our attention about fifty intestinal infections with cestodes, representing three genera and five species.

TÆNIA.

Of the fifty cestode infections about forty were with worms of the genus *Tænia.* Specific diagnosis from the ova alone was not attempted. Thirty-one specimens of adult *Tænia* have been deposited in the collection.

The cases given in the annual reports of the Superintendent of Government Laboratories and the statement given by Strong in 1901, above noted, appear to be the only mention, at least within recent years, of the presence of this genus in the Philippines.

Tænia saginata Gœze, 1782. Twenty-six of the thirty-one *Tænia* specimens are *Tænia saginata,* twenty-two being from native Filipinos; fourteen possess the scolex and strobila entire, while twelve were determined from the strobila alone. Two other specimens which were received probably belong to this species, but owing to poor preservation the characters could not clearly be made out and the identity is considered doubtful. One worm in the recent state presented a fairly distinct post-suctorial ridge around the scolex, resembling that described for *Tænia hominis* Linstow, 1902. Further examination failed to reveal any other characters described for that species and seemed to establish the specimen's identity with *T. saginata.* In all cases presenting any peculiarities which might throw doubt upon the determination, the specimens were studied in stained sections in comparison with material of known identity.

Tænia philippina sp. nov. The establishment of this species is proposed upon a single specimen obtained in 1905 by Mr. Hare of the Bureau of Science, from a prisoner in Bilibid Prison and placed in the museum of the Bureau of Science without determination. For specific diagnosis and description of type specimen, see pp. 542–550, figs. 1–17.

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[5] *J. Am. Med. Ass.* (1904), **42**, 891.

[6] *Bull. Hyg. Lab., U. S. Pub. Health & Mar.-Hosp. Serv.*, Wash. (1906), No. **28**, 50, 51.

[7] *J. Am. Med. Ass.* (1906), **47**, 685, 686.

Tænia solium Linnaeus, 1758. Two specimens of the pork tapeworm were obtained from native prisoners in Bilibid. Although the scolex was absent from each specimen, the size of the strobilæ, the massive uterine branches, not more than fifteen in number, the "third ovarian lobe" cut off by the vagina and the absence of extreme lengthening of the terminal segments seemed to render the diagnosis reasonably certain.

The proportion of the number of specimens of *Tænia saginata* (26) to the number of specimens of *Tænia solium* (2) here reported would seem to indicate with fair probability that the former is the much more prevalent of the two species; however, further work is necessary before the relative frequency of infection by these two species can be established with even approximate accuracy.[8]

HYMENOLEPIS.

Five infections with this genus have come to our attention, one in a native child under the professional care of Surgeon D. N. Carpenter, United States Navy, at Cavite, and four in adult males at Bilibid Prison, one of whom was Chinese and the others native. Parasites were recovered from two of the latter cases after treatment. Specific diagnosis of the child's infection was made, it is believed, with fair certainty, from the ova.

Hymenolepis nana (Siebold, 1852) Blanchard, 1891. One undamaged specimen of this species was taken from one of the Bilibid prisoners. The size of the ova obtained from Dr. Carpenter's case (from 40 by 35 μ to 57 by 42 μ, outer shell) and the absence of a granular layer between the outer and inner shells seemed fairly safe evidence that the infection was with *H. nana* rather than with *H. diminuta*. A careful study of the ova was not made in two of the infections with *Hymenolepis* and worms could not be secured.

It must be borne in mind in forming a judgment from the cases here reported regarding the frequency of infection with the dwarf tapeworm in the Islands, that in other countries *Hymenolepis nana* has appeared to show a marked tendency to infect children more frequently than adults[9] and that our work has been almost exclusively with the latter.

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[8] The results of meat inspection in Manila by the Bureau of Agriculture show a much higher percentage of cysticercosis in hogs than in cattle and I was led to expect a correspondingly greater prevalence of the pork tapeworm. However, I have been informed by Dr. Moberly, chief veterinarian, that most of the animals slaughtered in Manila are imported, nearly all of the native hogs and cattle being killed in the provinces, where meat inspection has not yet been established. The results of inspection, therefore, are not indicative of the relative prevalence of *Cysticercus cellulosae* and of *C. bovis* in native animals.

[9] Cima, Francesco: *Pediatria Napoli* (1893), **1**, 39; *Ibid.* (1896), **4**, 303; Ransom, Brayton H.: *Bull. Hyg. Lab., U. S. Pub. Health & Mar.-Hosp. Serv.*, Wash. (1904), No. **18**, 61; Stiles, Ch. Wardell, and Garrison, Philip E.: *Ibid.* (1906), No. **24**, 24.

Hallock's two infections with *Hymenolepis nana* in American soldiers recently returned from service in the Philippines and the two cases of American soldiers serving in the Philippines reported by Foster (see page 539) are, so far as we are aware, the only published reports for this species in the Islands. Through very indirect channels we have heard of one unpublished case found by a medical officer of the United States Army in one of the southern islands. Further, Dr. W. E. Musgrave informs us that he has noticed the ova several times in making fœcal examinations of natives and Americans during the past few years.

Hymenolepis diminuta (Rudolphi, 1819) Blanchard, 1891. A stool from a Chinese prisoner at Bilibid was brought to us with the diagnosis of *Hymenolepis nana*. However, the ova were very large, and fortunately all doubts regarding the specific identity of the parasites were removed by finding in the specimen from ten to twelve adult worms, from 3 to 6 centimeters in length, with a rudimentary, unarmed rostellum. These, upon further study of stained specimens, gave all the characters of *Hymenolepis diminuta*.

Unless cases have been published too recently to have come to our attention in Manila, this is the thirteenth infection with *Hymenolepis diminuta* reported for man. Unfortunately, the prisoner was discharged the day the specimens were passed and no history was secured. Therefore, it must remain an open question whether the infection was imported or contracted in the Islands. There are two other specimens of this species in the Bureau of Science Collection which were obtained from rats in Manila.

DIBOTHRIOCEPHALUS.

No infections in man with parasites of this genus have been found. It is mentioned in this connection because of the strong probability that it is present among the natives and because of the published opinion of at least one prominent helminthologist[10] that it may be found to be an important parasite in the Philippines. This expressed probability is based upon the great importance of fish in the diet of the natives, upon the proximity of Japan, where *D. latus* has been found in man, and upon the further fact that the presence in the Islands of at least one representative of *Dibothriocephalus* has been established recently by finding in the intestine of a domestic cat a worm of this genus which closely resembles, if it is not identical with, *D. latus* in its specific characters.

DIPLOGONOPORUS.

Diplogonoporus grandis (Blanchard, 1894) Luche, 1899, the double-pored, Japanese tapeworm, has also been mentioned as a possible Philippine parasite upon much the same grounds as *Dibothriocephalus*. Up to the present time it has not been observed.

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[10] Stiles, Ch. Wardell: *Ibid.* (1906), No. 25, 8.

SOMATIC CESTODE INFECTIONS.

No human infections with larval cestodes have been found. Strong,[11] reports two human infections with larval *Echinococcus* and upon inquiry we have learned of two or three cases of cysticercosis and of at least one other of echinococcus infection occurring in Manila within a few years. These verbal reports are all more or less indefinite and no specimens appear to have been preserved.

Two specimens of pork infected with *Cysticercus cellulosæ* have been received and this parasite appears to be rather common among the native hogs. The collection of the Bureau of Science also contains two specimens of adult *Echinococcus* [12] obtained from native dogs.

Tænia philippina sp. nov.

SPECIFIC DIAGNOSIS.—*Tænia* (*Tæniarhynchus*): Strobila 80 to 100 centimeters in length; 1 centimeter in maximum breadth; composed of about 800 proglottides. Head cuboid, 1 to 1.5 millimeters in diameter: suckers 0.35 by 0.40 millimeter; their lumena cylindrical to conical, directed forward and outward, 0.22 to 0.26 millimeter deep by 0.19 to 0.21 millimeter broad at their bases. Neck segmented, its minimum transverse diameter, 0.70 millimeter, at about the 75th segment and about 3 millimeters from posterior border of head. Sexual maturity first present at from 15 to 18 centimeters from anterior extremity and in about the 470th segment. Sexually mature proglottides 4 to 5 millimeters transversely by 0.8 to 1 millimeter longitudinally by from 1 to 1.3 millimeters dorso-ventrally; genital pore very slightly posterior to middle of lateral margin; cloaca 0.32 millimeter deep; cirrus distinct, 0.1 millimeter long by 0.03 millimeter broad; cirrus pouch 0.38 millimeter long by 0.10 millimeter broad; vas deferens forms loop within cirrus pouch, is without vesicula seminalis, and ends in a dilated extremity before reaching median line; testicles 0.13 to 0.16 millimeter long by 0.06 to 0.08 millimeter in their transverse diameters; vagina without setæ and forms two spiral coils between cloaca and excretory canal; vitellogen gland greatly elongated transversely, lies close against transverse canal, filling posterior one-tenth of medullary space in median line, its extremities attenuated and extending laterally two-thirds the distance from median line to excretory canals; ovaries composed of two unequal, transversely elongated lobes which lie close against the vitellogen gland posteriorly and extend laterally through three-fourths the distance from median line to excretory canals; the smaller lobe, proximal to pore of segment, is bounded internally by the median line and anteriorly by the vagina; the larger, distal lobe extends anteriorly to transverse plane passed through genital pore, its anterior third projecting across the median line, overlapping anteriorly the median extremity of the opposite lobe and filling the space between the two limbs of the posterior V-shaped portion of the uterus; the uterine stem presents a V-shaped course in the posterior half of the segment with its apex to the pore side of segment, its posterior extremity lying

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[11] *Circulars on Tropical Diseases* (1901), 1, 22.

[12] *Echinococcus granulosus* (Batsch, 1786) Rudolphi, 1805 = *Tænia echinococcus* Siebold, 1853. (See Stiles, Ch. Wardell, *Bull. Hyg. Lab., U. S. Pub. Health & Mar.-Hosp. Serv.,* Wash. (1906), No. 25, 75 et seq.

in the median line between the ovarian lobes, its anterior limb turning directly cephalad in the median line at about the center of the segment and presenting, in the anterior half of the segment, a spiral formation consisting of from four to six distinct, closely wound coils. In the gravid segment, the uterus presents an exceedingly compact formation consisting of a median stem and numerous long, slender, dichotomous branches, separated by thin partitions and frequently overlapping one another dorso-ventrally. Terminal segments equilateral or slightly longer than broad, measuring from 6 to 8 millimeters in longitudinal and transverse diameters and 3 millimeters dorso-ventrally. Embryophore oval, 35 to 41 μ long by 26 to 35 μ broad.

CYSTICERCUS unknown.

TYPE HOST.—*Homo sapiens.*

TYPE LOCALITY.—MANILA, P. I.

TYPE SPECIMEN.—No. 140, Helminthological Collection, Bureau of Science, Manila, P. I.

DESCRIPTION OF TYPE SPECIMEN.

Length 82.8 centimeters: transverse and dorso-ventral diameters respectively; of head, 1.5 millimeters by 0.75 millimeter; of neck, 0.72 by 0.24 millimeter; of mature proglottides, 10 by 1.3 millimeters; of terminal proglottides, 6 to 8 by 3 millimeters. Indentations of lateral margins suggesting segmentation are present close behind suckers; distinct segmentation appears 1 millimeter behind suckers; greatest constriction of neck, 3.5 millimeters behind suckers. (Figs. 2 to 4.) Transverse diameters of segments greater than longitudinal diameters except in terminal proglottides which are equilateral or slightly longer than broad. (Figs. 1, 18.) Genital pores prominent, and slightly behind middle of lateral margins of segments. (Figs. 1, 16 to 18.) Surfaces and margins of segments extend posteriorly in an elongated, cuff-like projection overlapping the succeeding segment and are indented by transverse infoldings of the cuticle, which appear to be formed in part, and perhaps wholly, by longitudinal contraction of the specimen. (Figs. 1, 5 to 7.) Calcareous corpuscles absent.

Head.—Transverse diameter, 1.5 millimeters; dorso-ventral diameter 0.75 millimeter, the lesser, dorso-ventral diameter appearing to be due to flattening by cover-glass pressure. Other dimensions as follows: From tip of head to plane passed through anterior borders of suckers, 0.15 millimeter, to posterior border of suckers, 0.64 millimeter; approximate longitudinal diameter of head, 1 millimeter. Rostellum (fig. 3) without hooks and retracted completely within head, its musculature globular, 0.16 millimeter in longitudinal by 0.112 millimeter in transverse diameter; slight depression at tip marks opening of lumen of minute apical sucker. Suckers (figs. 2 and 3) well embedded in musculature of head at its dorso- and ventro-lateral borders with the longitudinal axis of their lumena directed forward and outward; transverse diameter, 0.34 to 0.37 millimeter; longitudinal diameter, 0.37 to 0.4 millimeter; lumena cylindrical to conical, 0.224 to 0.260 millimeter deep by 0.19 to 0.21 millimeter broad at their bases.

Nervous system.—Situated 0.8 millimeter from anterior extremity of the head and 0.15 millimeter behind plane passed through posterior border of suckers, a large cavity (0.25 by 0.20 millimeter) incloses what appears to be a contracted, globular nerve ganglion (0.16 by 0.11 millimeter). (Fig. 3.) The nerve trunks proceed posteriorly in a straight course, at first dorso-lateral and later directly lateral of ventral excretory canals; concomitant dorsal and ventral fasciculi not apparent; lateral nerve trunks rather crescentic on cross section and measure 0.025 millimeter transversely by 0.150 dorso-ventrally in mature segments.[13]

Excretory system.—The ventral excretory canals pass in a straight course from the head through the lateral borders of the strobila, at first ventrad to and later directly in the median saggital plane. They attain their maximum diameter in the first gravid proglottides (0.35 to 0.40 millimeter in median transverse plane) and present a wide dilation (0.45 to 0.65 millimeter longitudinally by 0.80 to 0.90 millimeter transversely and dorso-ventrally) at each segmental junction. (Figs. 7, 8, 16; 17.) The transverse canals decrease in size as they pass inward from the dilated portions of the ventral canals, are oval or diamond shaped on cross section (figs. 5, 10) and are situated at the extreme posterior borders of the proximal segments, it being impossible in either young, mature, or gravid proglottides, to detect any extension of the proximal segment posterior to the canal. (Figs. 5, 8, 13, 17.)

The dorsal excretory canals can be traced as loosely wound, spiral tubules, 18 to 20 μ in diameter, as far as the first sexually mature proglottides. The coils of the spiral extend through a space of from 0.15 to 0.25 millimeter transversely and dorso-ventrally and number five to six complete turns in each segment. (Figs. 5, 7.)

Genital organs.—The primordium of the vas deferens and vagina appears in about the 150th segment, which measures 0.72 millimeter transversely by 0.24 millimeter longitudinally, and is situated 12 millimeters from anterior extremity. At about the 300th segment (1.75 millimeter by 0.45 millimeter, 2 centimeters from anterior extremity) the uterine stem is present as a distinct tract beginning posteriorly in the bulbous mass at the median extremity of the vagina, and passing diagonally anterolaterally toward the pore side of the segment until it reaches the angle between the vagina and vas deferens, which are now separated throughout the inner half of their course; thence turning at an acute angle, it takes an antero-median course to the median line, and along the median line to the anterior border of the segment. (Figs. 6, 7.) In the 400th segment (3 millimeters by 0.64 millimeter, 6 centimeters from anterior extremity) the female organs have became differentiated into the ovarian lobes and vitellogen gland, the testicles are appearing in lateral fields of medullary layer, the cloaca is forming at the lateral margin, the V-shaped course of the uterine stem in the posterior half of the segment is more sharply defined and its anterior prolongation in the median line is coiled in one or two loose spiral turns. The 475th proglottid is sexually mature. (Figs. 8, 10.)

Sexually mature proglottid.—Measurements: 5 millimeters transversely by 1 millimeter longitudinally by 1.35 millimeters dorso-ventrally, situated 15.5 centimeters from anterior extremity of specimen. Genital pore slightly posterior to middle of lateral margin (fig. 8); cloaca 0.32 millimeter deep (figs. 8, 12, 15); cirrus 0.1 millimeter long by 0.03 millimeter broad (fig. 15); cirrus pouch 0.35 millimeter long by 0.1 millimeter broad (fig. 15); vas deferens makes distinct

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[13] In the absence of specific staining for the nerve tissues, the nature of the globular structure in the posterior part of the head can not definitely be determined. The interpretation here made is based upon the marked similarity in appearance and structure in cross section between the lateral nerve trunks and the body in question when stained with carmine, upon the faint tracings of what appear to be nerve trunks arising from it, upon its position, and upon the elimination of other possible interpretations. The presence of a large, globular, well-defined ganglion as the center of the nervous system instead of the usual commissural arrangement of fibers would be an important character, but in view of the uncertainty of interpretation, it is not included in the diagnosis.

loop within cirrus pouch (fig. 15), is extremely convoluted after passing ventral canal, and ends shortly before reaching median line in distinct dilation (0.075 millimeter broad) which receives numerous vasa efferentia; testicles (figs. 8, 9, 13) measure 0.13 to 0.16 millimeter in length (dorso-ventral diameter of segment) by 0.06 to 0.08 millimeter in their transverse diameters.

Vagina makes two spiral coils before reaching excretory canal (figs. 11, 12) and after passing dorsad of the canal turns sharply ventrad, sweeps posteriorly, and near the median line it dilates to about twice its former diameter to form a muscular receptaculum seminis (0.040 millimeter in diameter) which abruptly enters a large globular chamber (0.16 millimeter in diameter) situated ventrally in the median line between the median extremities of the ovaries and the vitellogen gland and containing immature ova. (Figs. 7, 8, 13, 14.) From the posterior border of the chamber a tubule, 0.05 millimeter in diameter, passes dorsally in a convoluted course, taking up the vitellogen and shell gland ducts, and empties in the dilated posterior extremity of the uterus.

The vitellogen gland (figs. 8, 10, 13) lies along the extreme posterior border, close against the transverse canal, extending laterally two-thirds the distance from the median line to the ventral canals (1.2 millimeters). On longitudinal section in the median line it measures 0.3 millimeter dorso-ventrally by 0.01 millimeter longitudinally, filling the posterior one-tenth of the medullary space. Its lateral extremities are attenuated and lie entirely ventrad to the median saggital plane.

The ovary (figs. 8, 10, 13) is composed of two unequal, transversely elongated lobes with their posterior and ventral surfaces lying close against the vitellogen gland and the ventral boundary of the medullary layer respectively. Each lobe extends laterally three-fourths the distance from the median line to the ventral canals and is thickest in the posterior half of its median third where it completely fills the medullary space dorso-ventrally. The dorsal surfaces of the lobes are convex, approaching the ventral boundary of the medullary space at their median, anterior, and lateral margins.

The lesser lobe, proximal to the pore side of segment, is bounded anteriorly by the course of the vagina, except in its median third where, as it approaches the median line, a few filaments may pass ventrad to the vagina and reach a more anterior position.

The greater lobe, distad to the pore, extends anteriorly to or slightly beyond a transverse plane passed through genital pore; its anterior third is extended across the median line, slightly overlapping anteriorly the inner extremity of the opposite lobe.

The uterus (figs. 8 to 10) begins posteriorly as a pyriform dilation, 0.45 millimeter in diameter, situated obliquely between, but slightly dorsad to, the median borders of the ovarian lobes; diminishing in size the uterine stem passes obliquely antero-laterally toward the pore side of the segment until it reaches a point posterior to the median extremity of the vas deferens, when it turns inwardly at a sharp angle to its former course and, passing around the extended extremity of the greater ovarian lobe, continues obliquely forward and inward to the median line, which it reaches at about the center of the longitudinal axis of the segment; it then turns anteriorly and, in a spiral course of from four to six closely wound turns, reaches the anterior segmental border. In the spiral anterior limb the diameter of the uterine tube is about 0.035 millimeter, while the spiral fills a space of from 0.080 to 0.130 millimeter, the spiral coils becoming wider as they approach the anterior border of the segment.

Further development of the uterus (figs. 13, 16 to 18).—In the 500th segment (fig. 13) the dilated posterior extremity of the uterine tube has enlarged to a chamber measuring 0.32 millimeter dorso-ventrally and antero-posteriorly, by

0.8 millimeter transversely, which nearly fills the posterior portion of the medullary space in the median one-sixth of the segment. This joins a second dilation of about equal dimensions situated at the apex of the V-shaped portion of the uterine tube. In the anterior half of the segment the original four to six spiral turns are still distinct. The dilated, outer walls of the coils fill the dorso-ventral diameter of the medullary space, extend 0.4 millimeter on each side the median line, and project prominently forward into the transverse excretory canal. A number of shallow diverticula have grown out from the lateral walls of both the two posterior cavities and of the anterior spiral.

In segments 550 to 560 (fig. 16) from five to seven diverticula or branches have grown out from the lateral walls of the two posterior uterine dilations, extending laterally 1.17 millimeters or four-fifths the distance from the median line to the ventral canals. In the anterior half of the segments the original four to six coils of the spiral formation are distinguishable, and extend laterally 1.30 millimeters from the median line, or about two-thirds the distance to the lateral canals. The anterior extremity of the spiral has expanded laterally into a cavity which encroaches upon the lumen of the transverse canal. As they extend toward the lateral borders, the anterior coils and the posterior diverticula apparently by reason of mutual pressure and unequal growth, have more or less lost the regularity of their earlier structure, some pressing toward the dorsal and others toward the ventral surface and some are inclosed at their lateral extremities by two adjacent and more laterally extended outgrowths. As a result of this irregular development, saggital and longitudinal sections in different planes present widely differing pictures, resembling in some cases the uterine branches extending laterally from a median stem as in other species of *Tænia* and in others giving the appearance of the egg-sack formation found in other genera.

As a whole, the structure is remarkably compact, the ovic chambers being closely pressed together and the partitions between them extremely thin.

In the terminal proglottides (figs. 17, 18) the uterus fills the entire medullary space, encroaches upon the transverse and longitudinal canals, and short diverticula even press outward the muscular tissue of the cortex. A few of the outgrowths reach the lateral canals without division and in the same plane. Usually, their lateral extremities divide into two or three secondary branches, which may again divide, the divisions taking place in any plane, but usually antero-posteriorly. In a median saggital section it is sometimes possible to follow a single branch from the median line, or even slightly across it, to the lateral canal, but in most cases it passes dorsad or ventrad and its further lateral course can be followed only in sections in other planes. Occasionally, a branch ends considerably short of the lateral canals, other branches closing in around its lateral extremity.

Ova.—An outer shell could not be distinguished in either young or matured ova. The embryophore is oval, with a thick, radially striated shell. Measurement of fifty embryophores gave the following results in microns:

32.5×30	37 ×30	35 ×31.5	38.9×33.3	37 ×33.3
37 ×31.5	40 ×31.5	36 ×29.6	37 ×33.3	37.5×33
37.5×29.6	38 ×31.5	38 ×28	40.7×33.3	36 ×28
36 ×27	37 ×33.3	37.5×26	40.7×33.3	37 ×31.5
38 ×28.5	40.7×33.3	38 ×33	37 ×28	37 ×34
39 ×29	40 ×33	40.7×33.3	36.5×29.6	37 ×32
37 ×29.6	38.9×35	37 ×30	37 ×30	38.8×33.3
40.7×32	35 ×29.6	37 ×33	40 ×29.6	40.7×33.3
37.5×33.5	34 ×29.6	40.7×33.3	38.5×29.6	37 ×29.6
37 ×29.6	38 ×33.3	37 ×30	37 ×27	39 ×33

Maximum dimensions, 40.7×35 μ; minimum, 35×26 μ; average, 37.8×31.1 μ.

DISCUSSION.

The type specimen, although preserved for about two years in formalin, was in good condition, except that it was considerably, although apparently uniformly, contracted. In the description of the specimen, actual measurements are given, but in stating dimensions in the specific diagnosis I have endeavored to make a safe allowance for this contracted condition of the specimen and for individual differences which other specimens may present. While the examination of further material may lead to changes in the diagnosis of the species, either by the elimination of some characters now included or by the addition of new ones, it is believed that the principal morphological features described, namely, the transversely elongated ovarian lobes and vitellogen gland, the V and spiral formation of the uterine stem, the compact structure of the gravid uterus, the loop of the vas deferens, the length of the strobila, and the relative length and breadth of the individual segments, form a combination of characters which could neither be accounted for by peculiarities in the living individual nor by its post-mortem distortion.

We have, upon the presence of the rudimentary unarmed rostellum, placed the species tentatively in the subgenus *Tæniarhynchus* Weinland, 1853, pending the examination of the material by a systematic zoölogist.

ILLUSTRATIONS.

[Fig. 1 is from unstained material. All other material was stained with acid carmine, picric acid being used in some cases for counterstaining. Sections vary from 25 to 250 μ in thickness. The magnification was governed with the view of bringing out as well as possible the desired structures, rather than by the consideration of uniformity.]

PLATE I.

FIG. 1. Portion of strobila. Natural size.

2. Section of head through two suckers and border of neural cavity. 1×75.

3. Section of head in different plane, showing (?) neural cavity and ganglion, retracted rostellum and traces of excretory canals. 1×75.

4. Longitudinal section through neck, showing beginning of segmentation. 1×30.

PLATE II.

FIG. 5. Longitudinal sections of segments 372, 373 just internal to ventral excretory canal, showing spiral course of dorsal canal. 1×80.

6. Segments 375 to 397, showing the early appe[illegible] of the uterus and genital pore. 1×10.

7. Saggital sections of segments 398 to 403, showing in [illegible]t planes the early appearance of genital organs. 1×12.

PLATE III.

FIG. 8. Saggital sections of segments 451 to 455, showing sexual organs about mature. 1×12.

9. The same, highly magnified to show the form of uterus in a single segment. 1×98.

10. Transverse section of segment, showing the coils of vagina between cloaca and ventral canal. 1×80.

11. Saggital section of a younger segment showing the same. 1×82.

12. Transverse section of segment showing genital pore, cirrus, and loop of vas deferens in cirrus pouch. 1×90.

PLATE IV.

FIG. 13. Saggital sections of segments 493 to 495 in slightly different planes, showing posterior dilations of the uterus and its anterior coils which have nearly reached their maximum expansion before dividing. The heavily stained vitellogen gland extending along the transverse canal, and the more lightly stained ovarian lobes lying closely anterior to it are at their greatest development. 1×20.

14. Saggital section of segment, in a ventral plane, highly magnified to show inner length of vagina as it enters globular chamber from which the ovospermatic duct proceeds posteriorly toward the vitellogen gland and turns dorsally toward the shell gland which lies in a deeper plane. 1×40.

15. Longitudinal median section of segment 475, showing below, the vitellogen gland resting on the transverse canal; above and to the left the shell gland, above which the posterior extremity of uterus is faintly visible; more anteriorly the inner extremity of the greater ovarian lobe appears as it crosses the median line; above it are seen the well-expanded coils of the uterus. 1×60.

PLATE V.

FIG. 16. Saggital section of segments 555 to 556, showing further development of the uterus; numerous diverticula have spread laterally, in different planes from the outer walls of the uterine dilations and spiral coils. 1×10.

17. Saggital sections of segments 647 to 648 showing the irregular, compact structure of the uterus in gravid segments. 1×12.

18. Segment slightly compressed, showing gravid uterus. 1×10.

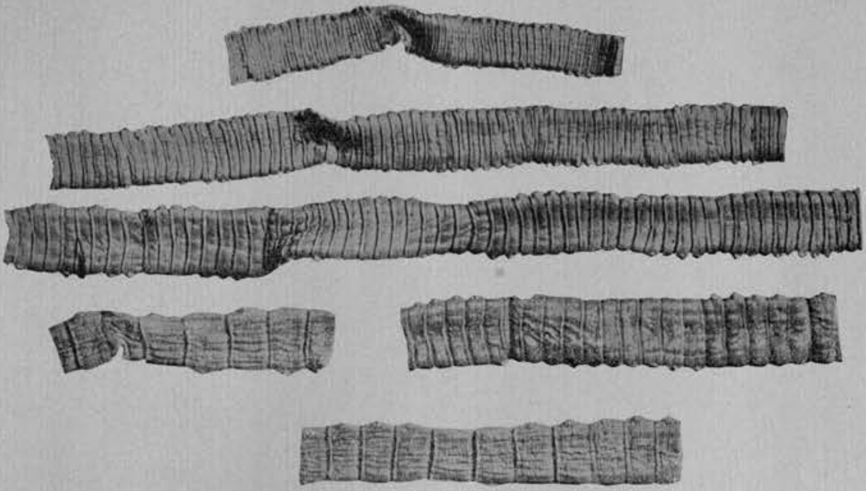

FIG. 1.

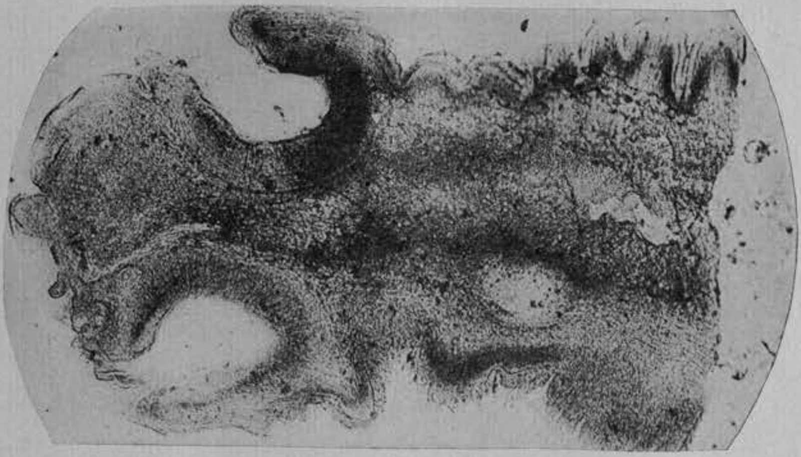

FIG. 2.

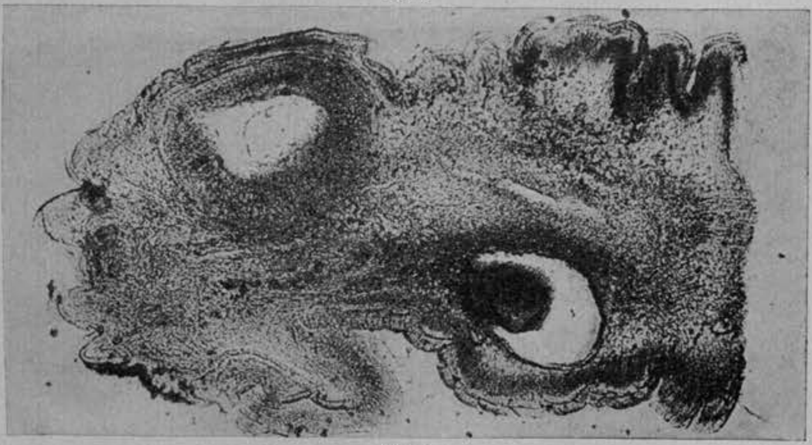

FIG. 3.

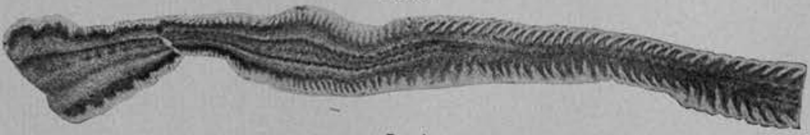

FIG. 4.

PLATE I.

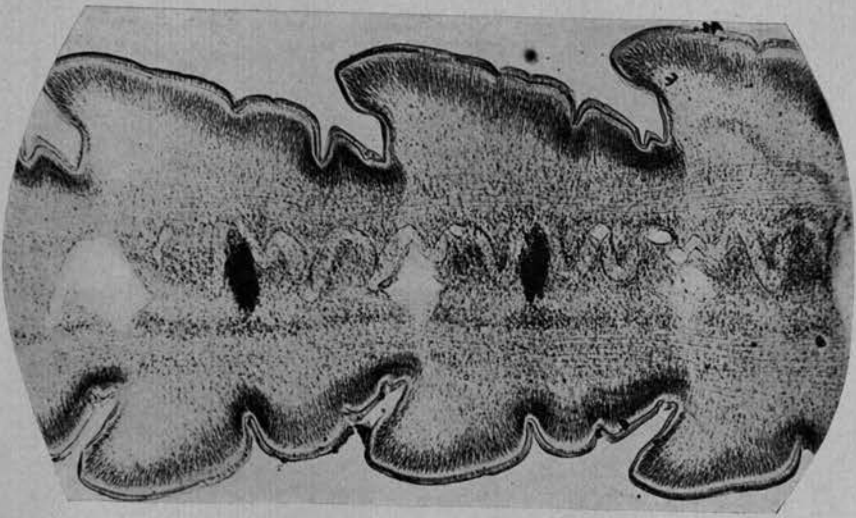

FIG. 5.

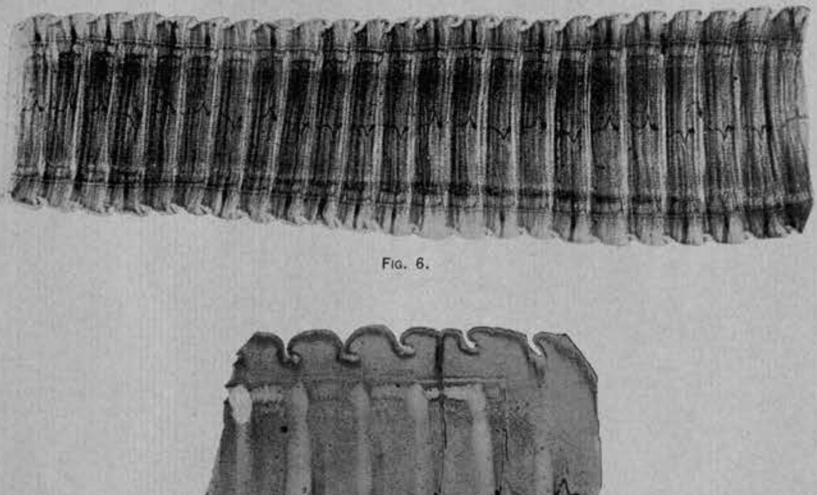

FIG. 6.

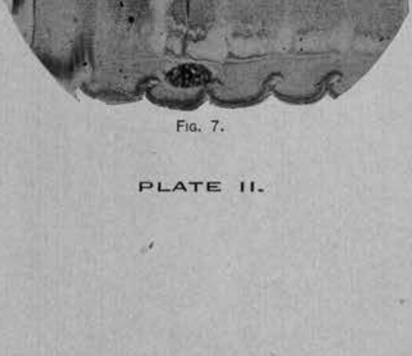

FIG. 7.

PLATE II.

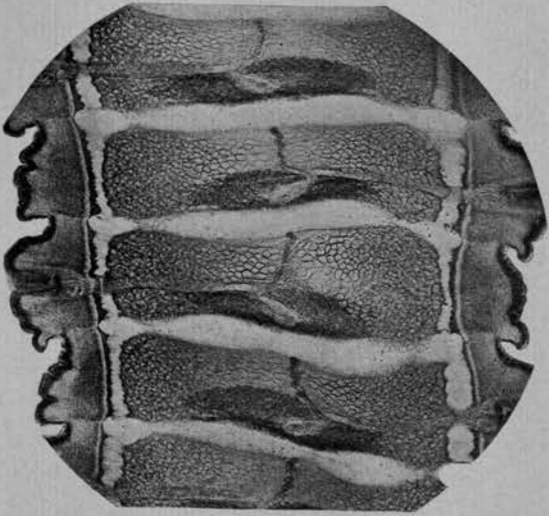

FIG. 8.

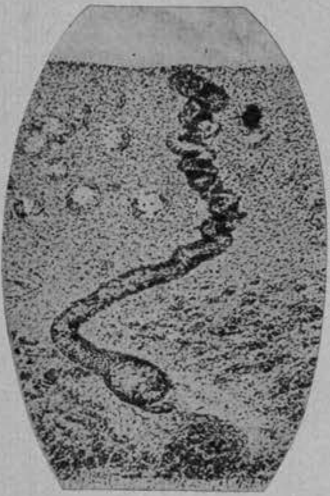

FIG. 9.

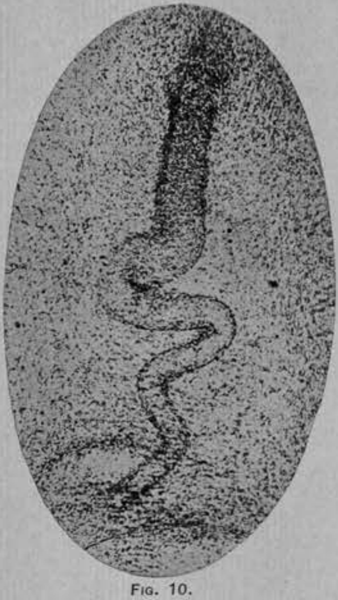

FIG. 10.

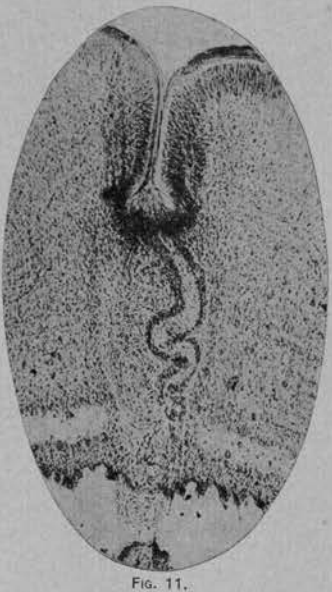

FIG. 11.

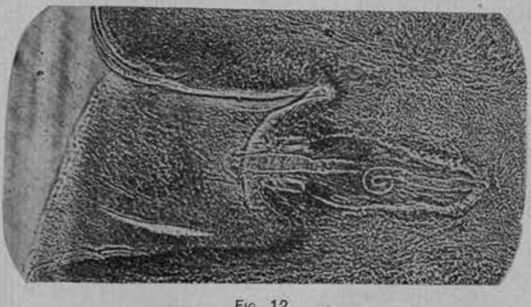

FIG. 12.

PLATE III.

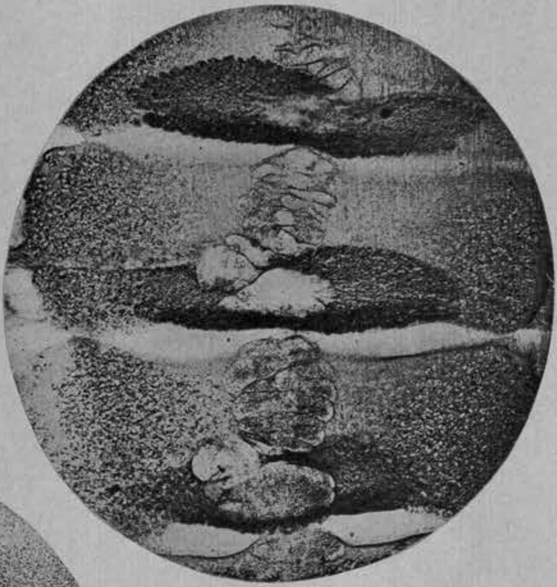

FIG. 13.

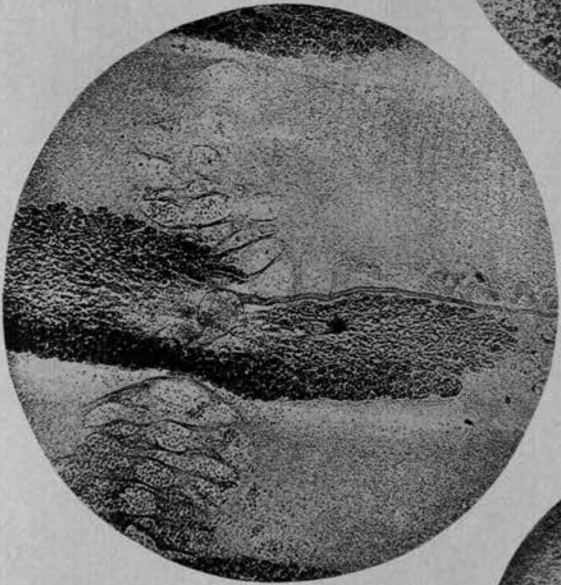

FIG. 14.

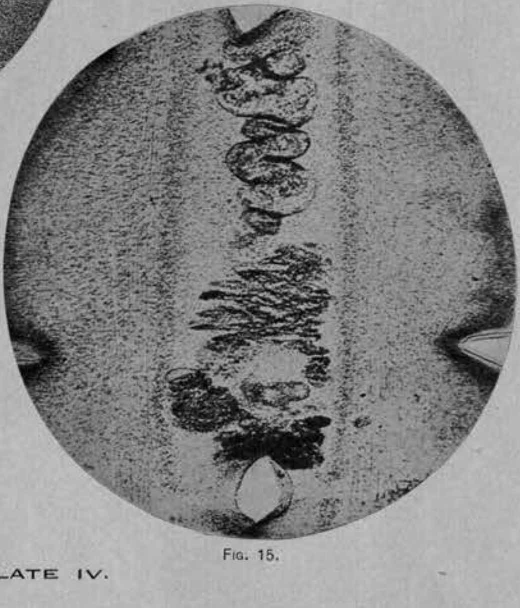

FIG. 15.

PLATE IV.

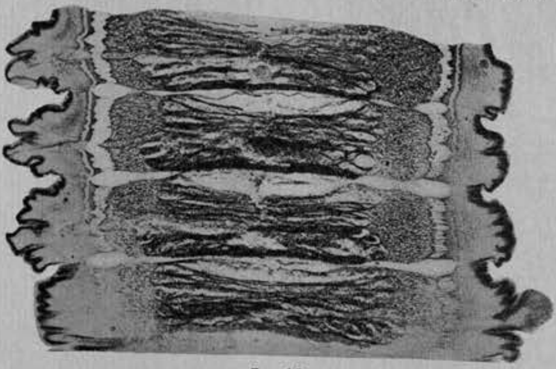

FIG. 16.

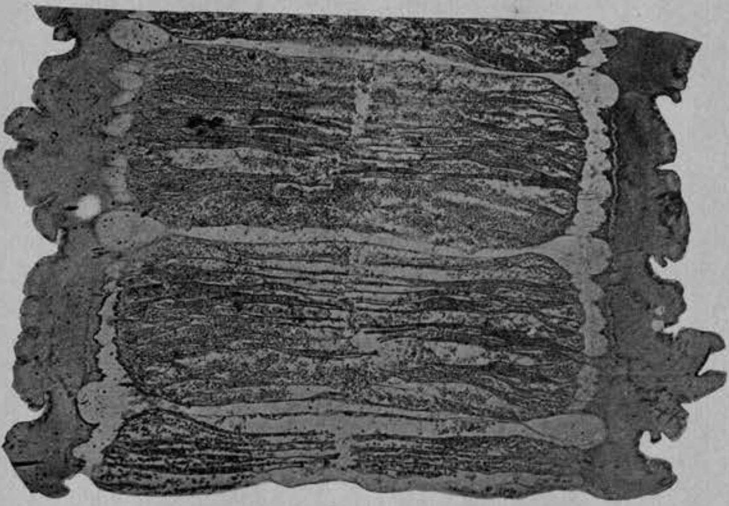

FIG. 17.

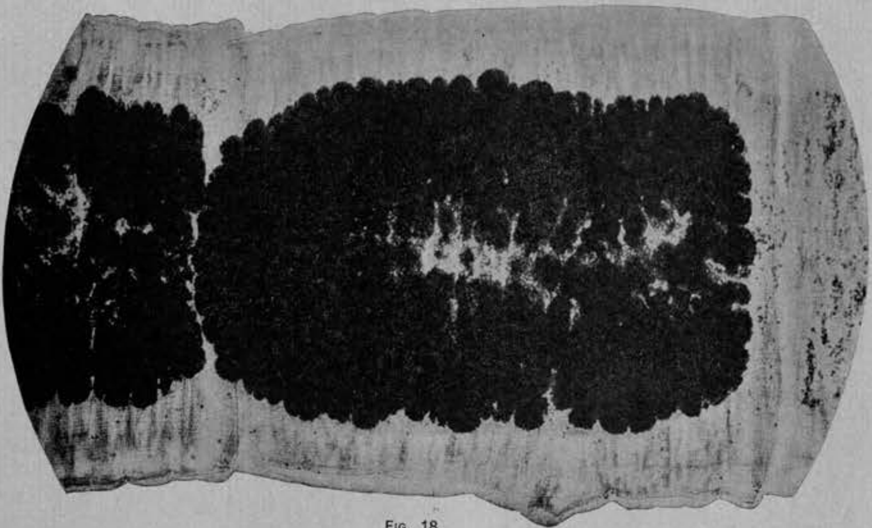

FIG. 18.

PLATE V.

NOTES ON CHRONIC ULCERS OCCURRING IN THE PHILIPPINES.

By George Cheyne Shattuck.

(From the Biological Laboratory, Bureau of Science, Manila, P. I.)

On the occasion of a recent visit to Manila opportunity was afforded me to work in the biological laboratory of the Bureau of Science and I became interested in attempting to determine whether or not Oriental sore occurs in Manila and in investigating the etiology of tropical ulcerations in general, as the question of etiology at present is in a state of much confusion.

This paper, embodying the results of my investigations, is based on clinical notes and microscopic findings in thirty-four cases of chronic ulcerative processes. With the exception of one Chinaman, all the patients were Filipinos. Fifteen of them lived in or near Manila,[1] and nineteen in the neighborhood of Catbalogan, on the Island of Samar. The Catbalogan cases were found among a number of natives suspected of leprosy who were being inspected by Dr. Heiser, Director of the Bureau of Health. They were held under observation for one week, and the ulcers examined repeatedly. Leprosy was not present in any of the patients of this series. In the Manila cases, smears were taken from the exudate on the surface of the ulcer and from the deeper tissues exposed by the curette. A piece of tissue[2] was removed and sectioned in four of these cases. In all the Catbalogan cases smears were taken from the exudate on the ulcer and from blood or serum expressed from a needle puncture close to the lesion. Giemsa's(9) new stain was used on the smears and a variety of stains were employed for the sections.

Table I.—*Catbalogan cases.*

[r.=right; l.=left.]

No.	Location of ulcers and scars.					Other lesions.
	Legs.	Feet.	Arms.	Hands and shoulders.	Other places.	
1	Scar, r.	Scar, r.				Hypertrophic periostitis, tibia, r.; contracture of toes, r.

---

[1] Twelve cases are from Dr. Saleeby's clinic at St. Luke's Dispensary, two from the service of Dr. Dudley at St. Paul's Hospital and one from that of Dr. Edwin C. Shattuck at the hospital of Bilibid Prison.

[2] Tissue was first put in alcohol and subsequently treated with acetone and embedded in paraffin. Thickness of sections 4 μ.

TABLE I.—*Catbalogan cases*—Continued.

[r.=right; l.=left.]

No.	Location of ulcers and scars.					Other lesions.
	Legs.	Feet.	Arms.	Hands and shoulders.	Other places.	
2	Scars, r. and l.; ulcer, r.	Scar, l		Scars, shoulder; ulcer hand, r.		Amputation of second finger, r.
3	Scar, ankle	Ulcers, l			Soft palate and uvula gone.	
4	Ulcers, r. and l.	Ulcers, r. and l.				Two toes amputated, r.; contractures, knees, and foot, l.
5	Scars, r. and l.; ulcer, l.		Scars, wrists	Scars, shoulders and hands.		Finger amputated r.
[a]6						
7	Scars, r. and l.; ulcers, r. and l.		Scars, r. and l.		Scar, nose	
8	Scars, r. and l.; ulcers, ankles.		Scar, l			Two toes amputated, r.
9	Scars, r. and l.; ulcers, r. and l.	Scars, r. and l.	Scars, elbows			Contracture legs.
10			Scars, r. and l.; ulcers, elbow, l.			
11	Scars, ankle l.; ulcers, ankle, l.		Scar, l		Scars, forehead.	Bones of forearm half destroyed; l. epitrochlea.
12	Scars, r. and l	Scars, r. and l.; ulcer, r.	Scars, elbow	Scars, shoulders.	do	Bone destruction, forehead.
13	Scars, r. and l.; ulcers, r.		Scars, r. and l.; ulcer, wrist, l.	do	do	Do.
14	Scars, r. and l.; ulcers r. and l.	Scars, r. and l.	Scars, elbow	do	do	Do.
15	Scars and ulcers, l.; scars knees.	Scars, r	Scars, l.; ulcers, l.	Scars, shoulder, l.	Scars, cheek	
16	Scars and ulcers, ankle r.; scars, l.					
17	Scars and ulcers, l.					Contracture, foot, l.
18	Scars and ulcer, r.					Hypertrophic periostitis, tibia l.; hand swollen and deformed.
19	do	Scars and ulcers, r.				Hypertrophic periostitis, tibia l.

[a] No. 6 was not tabulated.

TABLE II.—*Location and character of lesions in Manila cases.*

[r.=right; l.=left.]

No.	Legs.	Other places.	Miscellaneous.
[a]14319	Ulcer, ankle, r		
[b]2697		Ulcer, perineum, groin, penis.	General glandular enlargement.
[c]6536-3-P	Scars, r. and l.; ulcer, l		
[a]14612		Ulcer, buttock, l	
[a]14584	Scars, r. and l.; ulcer, r	Inguinal glands	
[b]1495	do	Scars, vulva	
[a]14125		Scars, foot and arm, r	
[a]11778	Scars, r. and l.; ulcer, l	Ulcer, palate	Hypertrophic periostitis, tibia, r.
[a]14413	Scars, r. and l.; ulcers, r. and l.		
[a]14518		Scars and ulcer, foot, l	
[a]13842	Scars, r. and l.; ulcers, r. and l.		
[a]12846		Ulcer, sole of foot	
[a]14560	Ulcer, ankle		
[a]13647	Ulcer, l		
[a]14581		Scars and ulcers, foot, l	

[a] St. Luke's Hospital number. [b] St. Paul's Hospital number. [c] Bilibid Hospital number.

Strong(1) described three very different types of ulcer found in Manila, exclusive of those due to tuberculosis, leprosy or syphilis. His first two cases can best be discussed in relation to the first and second cases described below. His third variety, observed in whites only, was not found in this series. The report of cases follows.

FIRST TYPE OF ULCERATION.

CASE I.—Native,[a] age 30, a laborer. Neither the patient nor his family have previously had any similar disease. Patient stated that four years ago, without assignable cause, a small boil appeared on his foot, that it burst spontaneously and did not heal. When he began coming to the dispensary, a month before I saw him, a diagnosis of phthisis was made. The patient denied that anything had ever been done except to apply dressings but when Dr. Saleeby went on service about that time he removed a stitch which was in the base of the ulcer. The patient was fairly well nourished, had no scars suggesting syphilis, and only the one external lesion. This was situated just below the external malleolus on the right foot where there was an ovoid swelling 2.5 × 2.5 × 0.75 centimeters in size, with a gash across it that went down nearly to the periosteum. The walls of the gash were somewhat ragged and the lower side was undermined. A little, yellowish slough was adherent to the base and sides. There was scarcely any discharge. The superficial skin over the swelling was thick and rough. The subcutaneous tissues were thickened. The swelling was firm. There was no tenderness, redness nor pigmentation. The lesion was curetted and a small piece excised for examination. The wound healed rapidly under simple dressings.

[a] St. Luke's Dispensary, No. 14319. First seen March 30, 1907.

A smear from the scrapings showed many polymorphonuclear leucocytes, a few large and small basophiles and occasionally a plasma cell. A very few cocci were observed, but no other bacteria nor parasites. In addition, a few round bodies identified as blastomyces by their contour and budding were evident in the smear from the exudate. A section stained with hæmatoxylin and eosin showed all the layers of the skin and a little subcutaneous tissue. Ulceration, infiltration, and necrosis of the upper layers of the skin appeared at one edge of the section. There was an increase of dense, fibrous tissue in the deeper parts of the sections and strands of this tissue ran out to the edge of the ulceration. The tissue near the ulceration was œdematous. Coagulation-necrosis, deposit of fibrin and considerable infiltration with small round and plasma cells and a few polymorphonuclear leucocytes was present. Other sections were stained for tubercle bacilli, as well as by the methods of Wright, Giemsa, Gram-Weigert, and silver impregnation. A few Gram-staining cocci were seen near the edge of the ulcer, but no blastomyces.

This lesion corresponds pretty closely to Strong's description of his "ulceration of the first type." On the one hand, the scarcity of pus is common to both, and the mode of onset is much the same; on the other, Strong encountered an oval blastomyces in the tissues, whereas in this case a round one was found sparingly in the exudate. It must be borne in mind that in this case the condition had existed for four years when it came under treatment, and that probably the lesion had been thoroughly curetted when the stitch was put in before specimens were obtained. Supposing it to be of parasitic origin, the parasites may either have died or have been scraped out. The duration of the ulcer in this instance was four times as long as is usual with Oriental sore. The finding of blastomyces in the exudate, which might have been secondary invaders, is of no value, and yet the assumption of a blastomycotic origin would explain the chronicity and mildness of the lesion better than any other diagnosis I can make.

SECOND TYPE OF ULCERATION.

CASE II.—Native, age 21.[4] Patient says that five months ago he had swellings in both groins which disappeared under treatment by a Chinese physician. There was also a sore on his penis. Then a small boil appeared in the right groin, resulting in an ulcer which spread slowly in spite of treatment. Another ulcer developed later between scrotum and thigh. The patient was treated for a month in the out-patient clinic and was admitted to the wards a month ago because the ulcers were obstinate. At the time of entrance he had a large chancroid on the penis. The chancroid has improved, but the large ulcer has remained in spite of vigorous anti-syphilitic treatment. On the contrary, several small ulcers have appeared recently near the anus.

The patient was fairly well nourished. He had no skin lesions other than those already mentioned. There was slight, general, glandular enlargement. The throat was negative and there was no periostitis. Extending from the base of the scrotum to the inner aspect of the right thigh was a smooth, granulating area neither elevated nor depressed and measuring about 10.5 centimeters in greatest diameter. Its outline was made up of curves like the arcs of intersecting circles. The margin was clearly defined, a little raised and slightly undermined. The surface of the granulations was level; they were boggy and dark bluish-red in color. No slough was visible. Several round, ulcerated papules appeared near the anus. Their interior resembled the surface of the large ulcer. There was an ulcer of moderate size in the groin which appeared to be improving. Dr. Dudley curetted the large ulcer and those near the anus. The granulations were so soft that they could almost have been wiped away. The fascia beneath appeared healthy. A week later the ulcer was covered with healthy granulation tissue, but at the end of the second week the surface was uneven and covered with small patches of grayish slough. The granulations at the edge were slightly exuberant, and those in the other portions appeared as if gnawed away. The color of the granulation was light red. The ulcer of the groin presented the same appearence. There had not been much change in the size of the ulcers.

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[4] St. Paul's No. 2697. First seen April 24, 1907.

Smears from the ulcerated surface showed ordinary pus and a few diplococci, but no other bacteria. Smears from the depths showed neither bacteria nor parasites. After curetting, agar cultures were made from the base in the ordinary way and under anaërobic conditions. They remained sterile. A culture on blood serum showed one colony of *staphylococcus* and several colonies of a white mould. A section stained with hæmatoxylin and eosin showed a rounded edge covered with epithelium and undermined by ulceration. The papillary layer of the epithelium was hypertrophied. The fibrous tissue of the corium was much increased and infiltrated with a few small round cells. There was a fibrinous exudate on the surface of the ulceration, having polymorphonuclear leucocytes and plasma cells in its meshes. Other sections were stained with eosin and methylene blue, by silver impregnation, by Gram's method, and for tubercle bacilli, but neither parasites nor bacteria were found in the tissues.

Like Dr. Strong's second case this ulcer began without previous injury, spread slowly in spite of treatment, and did not improve until after curetting. It differed clinically from Strong's case in having a smooth base and no slough, although the lesions may have been modified by the two months' treatment. The discoloration and bogginess of the surface recalls Scheube's (4) description of phagedenic ulcers. Against the supposition that this ulcer was due to the same organism as the chancroid, we have the fact that the lesions reacted differently to treatment, one healing and the other not. Syphilis is ruled out by the failure to respond to energetic treatment with iodide and mercurials. The appearance and behavior of the ulcer was not as it would have been if produced by tuberculosis. When first seen it differed from the description of ulcerative venereal granuloma (Menso and Manson) (2 and 3) in having a dull, bluish appearance, a smooth surface, a comparatively rapid development, and the absence of a tendency to heal in the center. Dr. Dudley is sure that until he curetted the ulcer it had neither been trimmed nor scraped nor cauterized, but only dressed. Its appearance was not that of ulcerative venereal granuloma. In favor of phagedena are the obstinacy of the ulcer, the discoloration of the granulation tissue, and the fact that common causes can be excluded.

THIRD TYPE OF ULCERATION.

CASE III.—Native,[5] age 37. The patient states that he had smallpox long ago, but no other illness or skin affection until two years previously when he received a wound from a board on the left leg. A large ulcer developed from the wound and at about the same time others appeared spontaneously on the other leg. These healed, but the primary ulcer did not. The patient was unusually well developed and well nourished. There were no lesions in the throat, no glandular enlargement and no anæsthetic areas nor infiltrated nodules.

On the anterior surface of the left leg, midway between knee and ankle, was a smooth scar about 15 centimeters long and 0.5 centimeter wide. The skin over the scar was pale, and the hair along its margin was white. On the external aspect of the leg, surrounded by pigmented scar tissue, was a shallow, granulating area. The edges were neither elevated nor undermined. The surface was clean. There was a small ulcer on the front of the leg, covered with a viscid, purulent exudate and a brownish crust. Grouped around the right ankle were many pigmented scars. The man was put on small doses of potassium iodide the day before I saw him and antiseptic dressings were used. When he was again seen six weeks later, the large ulcer had a border of new skin around the edge, and the granulations in the center appeared healthy. Two smears from the exudate of the small ulcer were examined. None were taken from the large ulcer because it had been cleaned. They showed polymorphonuclear leucocytes in abundance, tissue fragments and very numerous cocci and bacilli, some lying intra- and some extra-cellular.

The history and course of this case is typical of many others in which chronic ulcers developing after trauma have been followed by ulcerations in other parts of the body, particularly on the leg. It may be seen that lesions occurring in this manner are not all alike by referring to the second case described under the fourth type of ulceration. Some cases gave little indication of syphilis, but others had lesions suggesting this disease strongly. The second case[6] (Case V, p. 557) of the fourth type of ulceration is such a one.

Mense (3) states from personal observation that leg ulcers from trauma are very common in Africa among the negroes. He says that they heal without antisyphilitic treatment, are often large, but *generally single.* In our case the balance seems to swing toward syphilis as the most probable diagnosis, but there is much room for doubt. The bacteria observed were probably nothing more than secondary invaders. In favor of syphilis are the multiplicity of the lesions and their close resemblance to other lesions seen in undoubted syphilitics. Other characteristics are ambiguous.

[5] 6536-3-P, Bilibid Prison. First seen March 22, 1907.

[6] St. Luke's No. 14584.

FOURTH TYPE OF ULCERATION.

CASE IV.—Native,[7] coachman, age 33. Patient denied having had any skin lesion until three years ago when he was scratched by harness. An ulcer developed at the site of the scratch and gradually increased in size. The patient appeared healthy and showed none of the ordinary signs of syphilis. The lesion was roughly circular, 17 to 20 centimeters in diameter and occupied most of the posterior surface of the right buttock. It extended a short distance across the median line to the left buttock. The skin around the lesion appeared normal. The margin of the lesion was elevated and composed of a dense, inelastic tissue covered with pink, scaling epidermis, which was adherent and immovable. The same sort of tissue covered the greater part of the lesion. The ulcers near the margin were small, deep, pit-like and of uniform size. Their edges were firm, inverted and slightly undermined. They contained sero-purulent exudate and there was a yellowish slough at the base of each. In the central part of the lesion the ulcers were deeper, larger and serpiginous as if formed by confluence of smaller ones.

A smear showed polymorphonuclear leucocytes in fair numbers, a few large basophilic cells, and an occasional small lymphocyte and eosinophile. A few diplococci were observed but no other organisms. A section taken from the edge of an ulcer, stained with hæmatoxylin and eosin, showed thickening of the Malpighian and papillary layers of the epidermis and vacuolation of some of the cells of the upper *stratum lucidum*. The normal pigment in one place was wanting. The reticular stratum appeared œdematous and showed a marked increase of connective tissue cells. The lymph spaces were wide and contained polymorphonuclear leucocytes and plasma cells. There was no small round-cell infiltration about the veins and no endarteritis. The epidermis at one end of the section was undermined by ulceration. At this point traces only of the structure of the corium remained. There was much fibrinous exudate. Other sections stained by the Gram-Weigert method and for tubercle bacilli show many diplococci and a few bacilli in the horny layer but none in the deeper tissues. No tubercle bacilli were found and a section stained with silver showed no spirochætæ.

The patient was put on small doses of potassium iodide and the lesion was cleaned and covered with a wet bichloride dressing. It appeared much better after two days and at the end of six weeks the remnants of ulceration were covered with a few small scabs. The skin over the site of the lesion was still pink, but the subcutaneous induration had disappeared and the skin was soft.

CASE V.—Native,[8] age 55. History unreliable. The patient states that he was kicked by a horse seven months ago on the left leg, and that a chronic ulcer developed from the wound. The right leg became ulcerated at about the same time. The patient was poorly nourished. There were no scars in the throat nor any typical mucous patches. The inguinal glands were somewhat enlarged and hard, but the other glands were not. On the front of the left lower leg was a large, serpiginous, pocketed ulcer. The pockets contained moist, grayish slough and necrotic granulation tissue. The margin was indurated and bands of firm, inelastic tissue covered with adherent skin intersected the ulcer. The left leg presented a few pigmented scars around the ankle. The ulcer was cleaned and dressed with an antiseptic. The ulcer when seen again two days later appeared very much better. A piece of tissue was removed from one of the knobs in the base of the ulcer. The patient stopped coming and has not been seen since.

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[7] St. Luke's No. 14612. First seen March 22, 1907.

[8] St. Luke's No. 14584. First seen March 20, 1907.

Smears from scrapings showed blood, with a few polymorphonuclear leucocytes and many cocci and thick bacilli, the latter intra- and extra-cellular. One slide also contained a few blastomyces. A section stained with hæmatoxylin and eosin showed hypertrophy of the middle and papillary layers of the epidermis. There were a few polymorphonuclear leucocytes and plasma cells in the corium, but no lymphocytes around the vessels and no endarteritis. No necrotic nor ulcerated areas were seen. The silver stain and that for tubercle bacilli gave negative results. The Gram-Weigert stain showed many diplococci and bacilli in the horny layer (the former were positive and the latter negative to Gram).

CASE VI.—A young native woman.[9] The patient was poor and emaciated, the throat negative and the glands not enlarged. The lesions were confined to the legs and feet, which presented an appearance suggesting elephantiasis. The knees could not be completely straightened. The left foot was firmly held in the position of equinus by contracted, atrophied muscles. The right foot and ankle were much enlarged and held immovable by the dense, wooden character of the swelling. The skin was pink, unyielding and immovable with a macerated, scaling surface which merged into large ulcers encircling both ankles. There were sloughing, ragged ulcers of varying size and depth on the feet. The great and second toes of the right foot were absent, but the bone was not exposed. On the legs there were many small, round, punched-out ulcers of equal size, having firm, inverted edges and a yellowish slough at the base from which exuded a sero-purulent, sticky fluid. The legs were bathed in this foul-smelling exudate. They were not anæsthetic. This patient was under observation for a week. The lesions improved under simple washing.

Smears from the exudate taken on two occasions showed pus and enormous numbers of bacteria of many kinds. Spirochætæ were present in three out of four specimens and were very abundant in two of these. The fourth specimen contained blood with a little pus and only a few bacteria. Many of the spirochætæ could fairly be classed as *Sp. refringens* Schaudinn, but the characteristics of the majority were midway between these of typical *refringens* and typical *Treponema pallidum* Schaudinn. No typical examples of *pallidum* were seen.

No. 17 (see Table I) is an excellent example of the same sort of hypertrophy and ulceration. The toes on the affected limb were drawn up by contractures.

These four cases had two important common characters: First, a similar hypertrophic process affecting the skin in the same way; second, punched-out ulcers of the same type. These fundamental resemblances were noticeable at a glance. Stelwagon (6) in his description of ulcerating gummata says that an "elephantoid" condition with punched-out ulcers is common, that it has no clear limits and that in a few months it takes on a violaceous hue, softens, and breaks down or is absorbed. The indications are that the hypertrophic condition in the first and third cases has lasted much longer than the limit which this auther has given. The process in the first case was pretty definitely circumscribed, but its limits in the third case could not be determined. Chronic inflammation is shown in the sections. They are not characteristic of syphilis nor of tuberculosis. The bacteria and spirochætæ found in the smears probably were all secondary invaders, for the most part saprophytic. Their absence in sections points to this conclusion. If we concede that the four cases have the same etiology, we may say that they are due to a chronic, but curable, nontuberculous disease causing hypertrophy and ulceration, and that the disease is probably infectious. The diagnosis of syphilis is almost forced upon us. Blastomycotic infection and tertiary lesions of yaws are far less probable than syphilis. Elephantiasis does not require serious consideration as a possible diagnosis. Whether we do or do not concede that these cases have the same etiology, it must be recognized that they have many differences. The contracture and loss of toes in the third case and contracture in the fourth are not easily explained by assuming that syphilis alone is present. They may have resulted from long antecedent phagedenic ulcers which preceded the hypertrophic process. The present condition of the skin renders it difficult to judge of this by observation. Nerve leprosy seems less probable as an explanation in view of the careful consideration it was given and the negative verdict. Congenital defects and trauma are possible, but not probable, etiologic factors.

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[9] No. 4 of Catbalogan series. First seen April 10, 1907.

Table I of the Catbalogan cases shows (1) a strikingly large number of lesions on the legs, (2) multiplicity of lesions, (3) a marked resemblance in the distribution of lesions, and (4) a considerable number of deformities. Clinically, the resemblance between individual lesions in different cases and between individual cases themselves is very striking. This points to a common etiology.

Leprosy, tuberculosis, and syphilis require careful consideration in the diagnosis of this group. Against leprosy we have the facts that there were no signs such as loss of eyebrows, there were no nodules, spots, nor anæsthesia, and also six or more smears taken from inside the nose, the lobes of the ears and from the lesions in every case were negative. Were the bone lesions tuberculous, sinuses or typical tubercular lesions of the skin would have been present in some of the cases. Nothing of this sort was found and no tubercle bacilli were seen in the smears made for leprosy.

The following lesions: Destruction of soft palate, dactylitis, destruction of the phalanges, depressions in the bone of the forehead, and "elephantoid" swellings of the legs, with gumma-like ulcerations indicate the presence of syphilis. The microscopic evidence is negative. Syphilis, then, is a probable diagnosis, but syphilis, uncomplicated, rarely produces ulcerations deep enough to cripple limbs or amputate toes. According to Scheube, tropical ulcerating phagedena does this very thing. It commonly invades unprotected lesions in the Tropics. Therefore, the diagnosis of syphilis complicated by phagedena might be made.

TABLE III.—*Tabular summary of all cases.*

Location and character of lesions.	Number of each.	Percentage of each.
Ulcers or scars of lower extremity	32	94
Ulcers or scars of upper extremity	13	35
Ulcers or scars in other locations	14	41
General glandular enlargement	1	3
Hypertrophic bone lesions	4	12
Destructive bone lesions	4	12
Amputations	4	12
Contractures	5	15

Table II of the Manila cases shows the preponderance of lesions on the legs, as is the case in Table I, and also demonstrates that the number and variety of lesions in the Manila cases was much less than in those from Catbalogan. Their essential characters are the same.

The spirochætæ which were seen present some interesting features. The organisms were found in five of the thirty-four cases (about 15 per cent). There were several varieties which may be divided into three classes.

TABLE IV.—*Occurrence of spirochætæ.*

Case No.	Class A.	Class B.	Class C.
4	Numerous	Abundant	Rare.
8	Few	Numerous	
14	do	Few	
14560	do	Numerous	
12846	Numerous	Abundant	

Characteristics of Class A: 1. Outline wavy, rather than spiral. 2. Curves very large and sweeping. 3. Curves few, rarely more than six. 4. Body wide and short. 5. Body as a whole nearly straight. 6. Ends gradually tapering to a point. 7. Stains dark blue with Giemsa. 8. Stains heavily.

Characteristics of Class B: 1. Outline wavy rather than spiral. 2. Average length of curve is moderate. 3. Curves shallow. 4. Number of curves rarely more than ten. 5. Curves often of different sizes in same individual. 6. Body often bent, curved or looped. 7. Body of medium thickness and medium length. 8. Both ends tapered as a rule; sometimes one end is truncated. 9. Stains blue or purple with Giemsa.

Characteristics of Class C: 1. Outline wavy or spiral. 2. Curves very short. 3. Curves shallow. 4. Number of curves rarely more than fourteen. 5. Curves of nearly uniform size. 6. Body as a whole nearly straight. 7. Body very thin and long. 8. Ends tapered. 9. Stains light blue with Giemsa; sometimes purple. 10. Stains faintly.

Evidence of transverse division was observed in all three classes. It was indicated by a pale area between two curves or at the top of a curve near the middle of the organism (Pl. I, fig. 1, *a*), or at two points in the same organism, dividing it into thirds. The pale area appeared to be narrower than the rest of the body. This is particularly well shown in examples of Class A, when the division, if such it be, is nearly complete. It might be argued that two individuals happening to lie end to end would produce a false impression of transverse division, but against this assumption are the facts that many instances can be found in a single specimen and that the line of the curve is unbroken. These appearances occur, but they are by no means as distinct in Classes B and C as in Class A; similar ones have been described by Goldhorn (8) and Fox (5) for *Treponema pallidum*, and by Novy and Knapp (7) for *Spirochæta obermeieri*. Classes B and C also at times suggest longitudinal division or agglutination, or both. A very few examples were observed in which one end of the organism was distinctly forked. Two organisms, intertwined, were not uncommonly seen, but in many of these instances four separate ends could be distinguished, so that the arrangement might have come about either by longitudinal division or by agglutination. One large bundle of organisms of Class C was observed, the individuals being arranged nearly parallel to each other. One *spirochæta* projecting from the side of the bundle showed a forked extremity, with the junction of the ends entirely clear of the bundle where it could plainly be seen. As the number of specimens which I have examined is a small one, these data on the question of multiplication of *spirochætæ* are not considered to be sufficient to prove the occurrence of either form of division.

I could not establish the causal relationship between the *spirochætæ* and the ulcers.

SUMMARY.

Four different types of ulceration were studied. The first and second types were not definitely diagnosed. They were probably infections *sui generis.* In the third type the weight of evidence is slightly in favor of syphilis. Many examples of this were seen. In the fourth type the probability of syphilis is strong. The remaining cases of the series seem to be variations of types three and four, which might all be placed in one group. Together they would comprise 94 per cent of the series (thirty-two cases). The diagnoses were all made clinically, because the microscopical findings were negative or ambiguous.

The cellular content of the exudates was nearly the same in all the cases. The bacteria were as a rule also identical.

Blastomyces were found in the exudate from two cases, but were not proved to be of etiological importance.

Spirochæta refringens and two other varieties of *spirochætæ* were observed, but they were not believed to bear a causal relationship to the ulcers. Evidence of division was seen among the spirochætæ.

Microscopical findings in brief.

	Number of cases.	Percentage.
Pus and pyogenic bacteria	34	100
Spirochætæ	5	15
Blastomyces	2	6

CONCLUSION.

Oriental sore as described in the text-books, Madura foot, and the typical phagedenic ulcer of Manson and Scheube were looked for in vain. No varicose ulcers were seen. The proportion of ulcers due to typical phagedena, blastomyces, and infections *sui generis* is small. A very large proportion of the chronic ulcers are syphilitic. Owing to neglect, the lesions are unusual in degree if not in kind, and they become very destructive.

The view that *Spirochæta refringens* is a bacterium is supported by strong evidence of transverse division.

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ILLUSTRATIONS.

(Photomicrographs by Mr. Charles Martin, Bureau of Science, Manila, P. I.)

PLATE I.

FIG. 1. *Spirochæta refringens* (Class A) giving appearance of transverse divison nearly complete; also an unidentified spirochæta of Class B. (1,000 diam.)

2. *Spirochæta refringens* (Class A) showing signs of transverse division. (1,000 diam.)

3. Spirochæta of Class B, one of them showing a forked end suggesting longitudinal division. (1,000 diam.)

4. Twisted examples of Class B. (1,000 diam.)

5. Spirochæta of Class C, which approaches *Treponema pallidum*, side by side witn *Spirochæta refringens*. (1,000 diam.)

6. Spirochæta of Class C, undergoing division. Separation incomplete. (1,200 diam.)

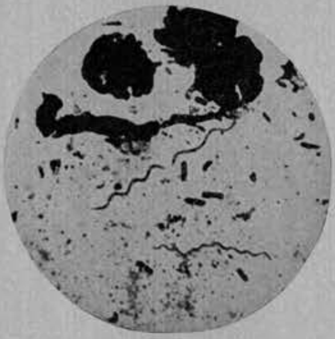

FIG. 1.

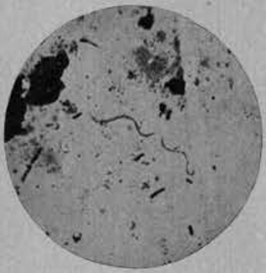

FIG. 2.

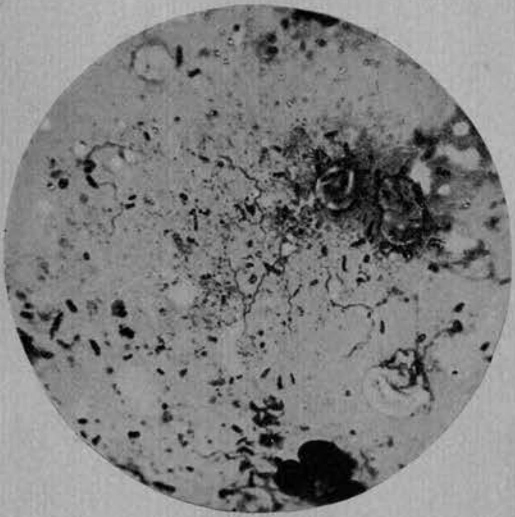

FIG. 3.

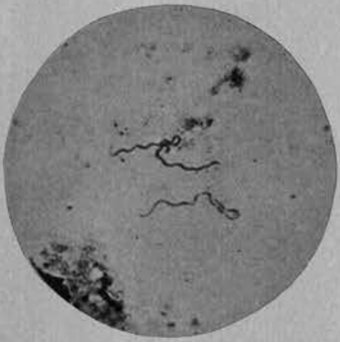

FIG. 4.

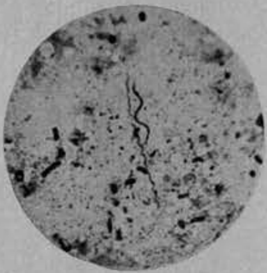

FIG. 5.

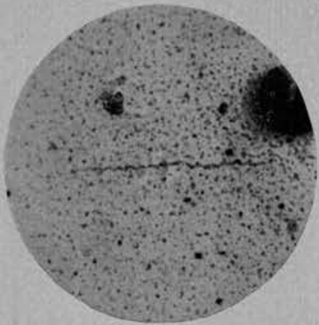

FIG. 6.

PLATE I.

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